Morphogenesis throughout saprobic and parasitic cycles of *Coccidioides immitis*

M. Huppert, S. H. Sun & J. L. Harrison
Audie L. Murphy Memorial Veterans Hospital and University of Texas Health Science Center, San Antonio, TX 78284, USA

Abstract

The fungus, *Coccidioides immitis,* differs from other dimorphic pathogens in that its parasitic stage is a complex morphogenic cycle, raising the question that changes in structure and composition during morphogenesis might influence host responses. As a prelude to examining the interaction of fungal morphogenesis and host responses, the life cycle of this fungus has been examined in greater detail than previously accomplished. During saprobic development, alternating enterothallic arthroconidia are formed as infectious propagules. The outer wall is broken and loosely adherent. Under in vitro conditions supporting the parasitic cycle, multinucleate arthroconidia transform into uninucleate round cells. Rapid, synchronous, nuclear replication is initiated, accompanied by increase in cell mass and deposition of new cell wall substance. As karyokinesis ceases, morphologic differentiation begins with invagination of the inner layers of the spherule wall and then is progressive, eventually segmenting the protoplasm into uninucleate endospores grouped in clusters within a hyaline membrane. Endospores, escaping through a break in the spherule wall, are held in aggregates by fibrils which are stretched and broken as endospores separate. It would seem that rapid production of hundreds of progeny from an original single cell, protected during development by an enclosing spherule wall and then released in clusters, should favor establishment of the fungus in a host, and dynamic changes in the cell wall during morphogenesis should influence the host response.

Introduction

Infection of animals, including man, is an accidental event in the natural life history of *Coccidioides immitis.* This fugus exists in the soil of semiarid regions of the Western Hemisphere and infection of animals is not a requirement for perpetuation of the species. *C. immitis* has a complete life cycle as a saprobe in its natural habitat (23), and this is reproduced in the laboratory on common culture media at either ambient or incubator temperatures. In nature and under usual laboratory conditions, the fungus develops a mycelial colony with different isolates varying in texture and pigmentation of aerial hyphae, gray to white being most common (17). Propagation of the species is achieved by formation of alternating enterothallic arthroconidia in the aerial hyphae. The terminology, arthroconidia replacing arthrospores, is used in accordance with the recommendation of the First International Specialists' Workshop and Conference on Criteria and Terminology in the Classification of Fungi Imperfecti (18). The arthroconidia secede readily from the thallus, and are dispersed by wind as the soil dries after the rainy season. The airborne arthroconidia are infectious for man and animals. In the host and under special growth conditions in the laboratory, arthroconidia transform into round cells which mature into spherules containing many endospores. Release of endospores initiates a second cycle of cells developing into endosporulating spherules, and this is repeti-

Mycopathologia 78, 107–122 (1982). 0301-486x/82/0782-0107 $1.20.

tive within the infected host. These characteristics are illustrated diagramatically in Figure 1.

This pattern of colony characteristics and microscopic morphology in the saprobic phase is not unusual (10, 28). Although many species of fungi resemble *C. immitis,* it is unique in three aspects. First, it is the only species among those forming alternating arthroconidia known to be a primary pathogen capable of producing systemic disease. Second, *C. immitis* is dimorphic; i.e., its saprobic and parasitic forms are morphologically distinct. The parasitic form, however, is not a yeast as most dimorphic pathogenic fungi, but attains its ultimate and characteristic expression as an endosporulating spherule. Third, the parasitic stage occurs in a morphogenetic cycle rather than a single morphologic form. Thus, *C. immitis* differs significantly from other saprobic and parasitic fungi.

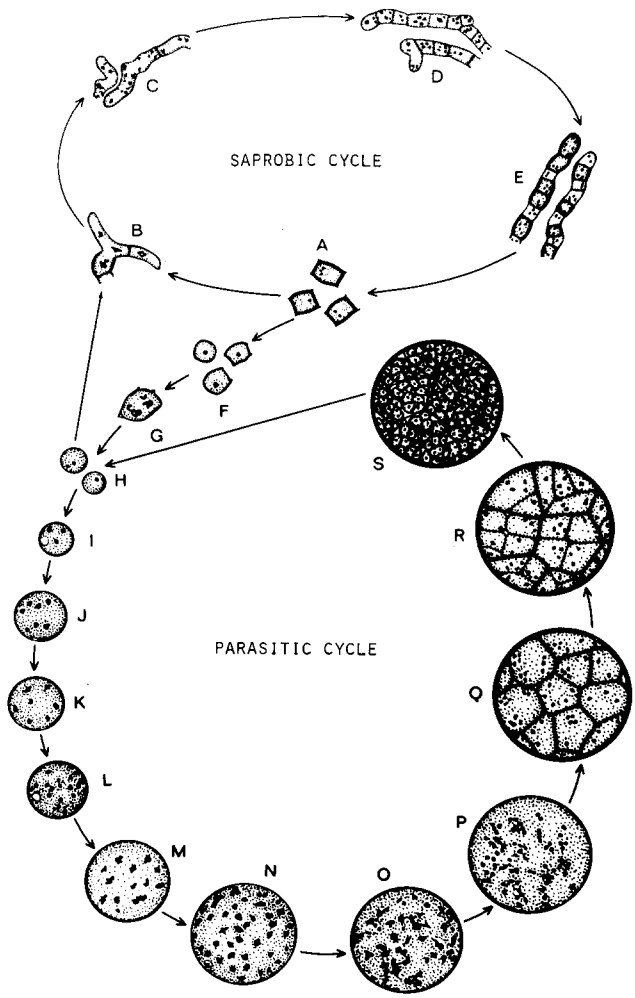


Fig. 1. Life cycle of *C. immitis* illustrating the saprobic cycle (A–E) and transformation (A, F–H) into the parasitic spherule-endospore cycle (H–S, H). (Reproduced from 33 by permission.)

Since *C. immitis* undergoes cyclical morphogenesis, it is pertinent to ask whether changes in structure and composition might affect host responses. There is some evidence supporting this. Liberated endospores attract an intense neutrophil response, but mature spherules are surrounded by monocytes and giant cells (11, 12). Immunogenicity is associated with the cell wall (19) and increases as cells mature from arthroconidia or endospores to spherules (21). As a prelude to studying the impact of morphogenesis on host response, we have characterized morphogenetic development throughout these cycles. Studies of nuclear cytology by light microscopy and of morphology by transmission electron microscopy (TEM) have been published by us (33, 34) and by others (3, 8, 20, 25, 26). Here we present our observations using scanning electron microscopy (SEM), collating these withour previous studies. We have focused on the cell wall as the primary site of interaction between host and parasite. Several morphogenetic features, some demonstrated for the first time, are considered for potential impact on host responses, including a loosely adherent outer wall layer on arthroconidia, rapid formation within a thick-walled shell of hundreds of progeny derived from a single cell, and release of these progeny held in clusters by a membrane and connecting fibrils. The saprobic cycle is included for three reasons: (a) this leads to the development of the infectious propagule; (b) most of the antigens used for studying the host response have been prepared from saprobic growth; and (c) the life history of *C. immitis* would not be complete without it.

Materials and methods

Four isolates of *C. immitis* were studied by SEM; Silveira (ATCC 28868), C60, C566, and C606. The first two are old isolates, obtained originally from humans but maintained in stock culture for many years, and the last two are recent clinical isolates. Cultures were grown on 1% glucose–0.5% yeast extract agar, and arthroconidia were harvested by the spinning magnetic bar technic described previously (33, 34).

Two procedures were used for obtaining cells to be processed for SEM. A slide culture method was used for making most of the preparations from both saprobic and parasitic stages. Round coverslips (12 mm diameter) were autoclaved, dried, precoated with a thin film of 0.1% Ionagar #2 (Oxoid), and dried thoroughly in a sterile container. These were pressed lightly on small agar blocks (3–4 $mm^2 \times 2.5$ mm deep) which had been inoculated on the surface with 0.2 ml of an arthroconidia suspension containing approximately 10^8 cells per ml. Two types of agar and nutrients were used to obtain optimal growth conditions for each cycle. The saprobic stages were cultivated on 1% glucose–0.5% yeast extract in 1.5% agar (Difco) and incubated at 30C. The parasitic stages were grown on modified Converse medium (21) in 1% Ionagar #2 and incubated at 40C in an atmosphere of 20% CO_2–80% air (Modular Incubator Chamber, Billups-Rothenberg). The slide culture assemblies were maintained in moist chambers. Coverslips with adherent growth and agar blocks were processed separately for SEM. The slide culture method is advantageous because stages of morphogenesis can be monitored microscopically.

The second method was used only for the parasitic cycle. Sterile filter membranes (13 mm, 0.4 μm pore size, Nuclepore) were placed on the surface of modified Converse medium in 50 × 12 mm box-type petri dishes (Falcon 1006). The membranes were inoculated and incubated as described above. Membranes were removed at intervals and transferred to a stainless steel Swinny filter holder (Gelman) to facilitate processing for SEM.

Specimens were fixed (6% glutaraldehyde and 1% OsO_4), washed in buffer (0.1 M sodium cacodylate, pH 7.1–7.4) and dehydrated (ethanol followed by amyl acetate in ethanol) according to the procedure of Cole and Samson (5). After critical point drying (Bomar SPC-900/EX), specimens were attached to aluminium stubs with conductive silver paint and coated with gold-palladium (60:40). The specimens were examined at 30 KV in an ISI-40 or Phillips 5000 scanning electron microscope. Selected stages in both cycles were photographed with Polaroid Type 55 Positive/Negative Film (9 × 12 cm), and the negative was printed on Kodak Polycontrast Rapid II RC paper.

Results

The principle morphological events for all four isolates were essentially the same, and, except where indicated, the following descriptions apply to all isolates.

Saprobic cycle

The cell wall of the harvested arthroconidium (Fig. 2) has two main components: an outer electron opaque layer and an inner electron translucent layer, approximately 0.1 μ and 0.2 μ in thickness, respectively. These will be referred to as the outer wall (OW) and inner wall (IW) although the latter is, and the former may be, multilayered. The IW envelops the arthroconidium completely, but the OW is a remnant of the wall from the parent fertile hypha and adheres to the arthroconidium in irregular folds. The intrahyphal ends of the cell are recognized by the broken tags of OW projecting beyond the arthroconidium. When cultured under conditions favoring mycelial growth, one or more germ tubes are formed (Fig. 3). There is no evidence for polarized growth since germ tubes may penetrate the wall either at the intrahyphal ends or through the sides of the arthroconidium. The OW may be shed or remain adherent, but the innermost layers of the IW are continuous along the advancing germ tube. Nuclear replication occurs early and the nuclei flow into the germ tube. The mycelium is composed of septate hyphae (Fig. 4) with a simple septal pore and Woronin bodies, indicating an ascomycetous relationship. Differentiation of fertile hyphae into arthroconidia is initially apparent as alternating light and dark areas by SEM (Fig. 5A). The light areas are arthroconidial initials with thickening walls, and the dark areas are thin walled, intercalary cells destined to degenerate (5). Lomasomes (Fig. 5B) are apparent in the young fertile hyphae, and their spacing approximates septum formation. Maturing arthroconidia (Fig. 6) are separated from adjacent cells by a multilayered transverse septum derived from the IW of each cell (i.e., a double wall), but the OW is continuous along the hyphal filament and does not participate in the new wall enclosing the arthbroconidium. Separation and secession of the matured arthroconidium (Fig. 7) occur following autolysis and wall collapse of the intercalary cell. Production of arthroconidia

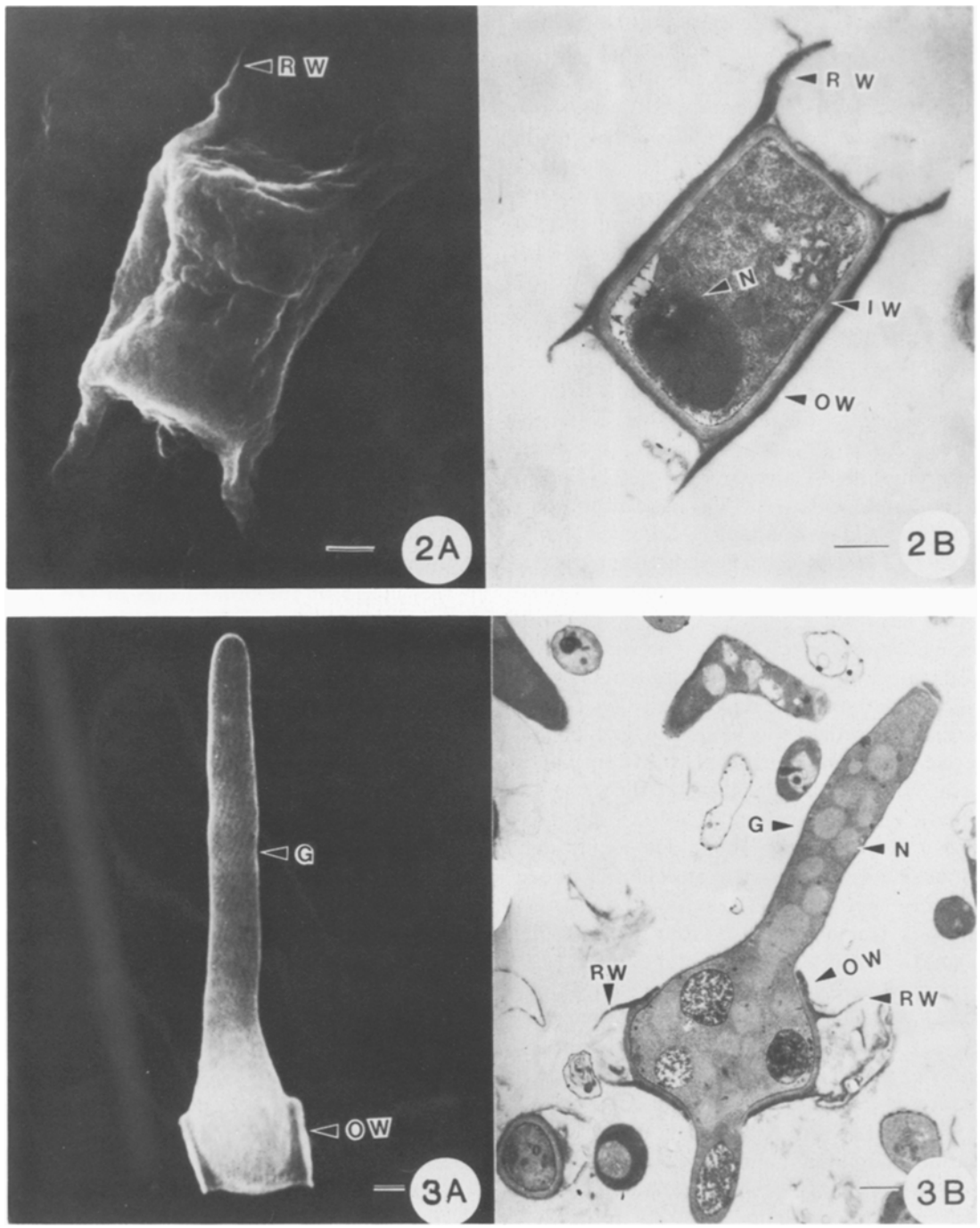


Fig. 2. Arthroconidium separated from parent fertile hyphae. IW, inner wall layers; OW, outer wall layers; RW, remnants of OW of disintegrated cell; N, nucleus. Bars = 1 μ. (Figure 2B reproduced from 34 by permission.) *Fig. 3.* Germinated arthroconidium. G, germ tube; IW, OW, RW, and N as in Figure 2. Bars = 1 μ. (Figure 3B reproduced from 34 by permission.)

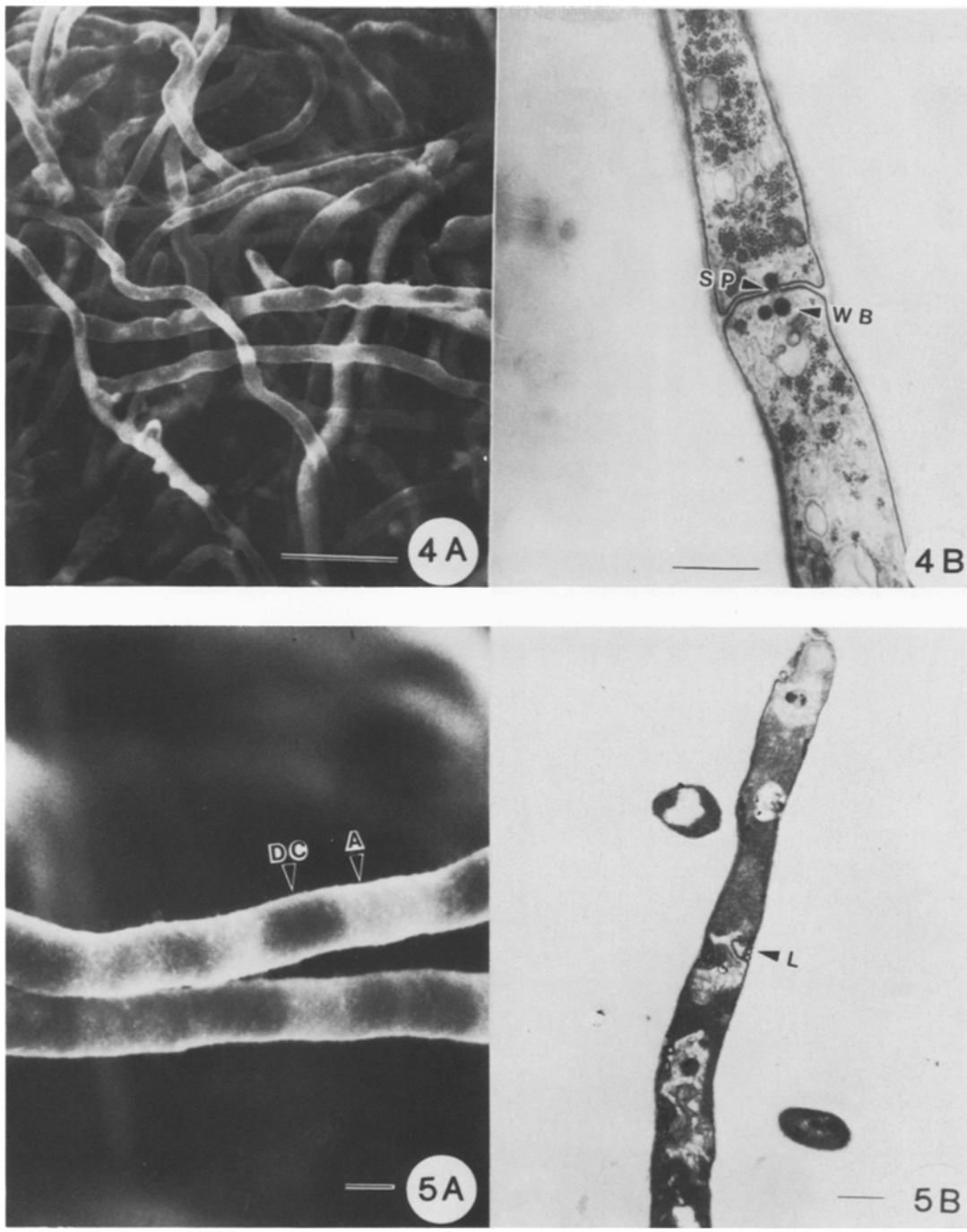


Fig. 4. Mycelial development. SP, septal pore; WB, Woronin body. Bars = 10 μ in A and 1 μ in B. (Figure 4B reproduced from 34 by permission.) *Fig. 5.* Development of fertile hyphae. A, arthroconidial primordium; DC, disintegrating cell; L, lomasome. Bars = 1 μ. (Figure 5B reproduced from 34 by permission.)

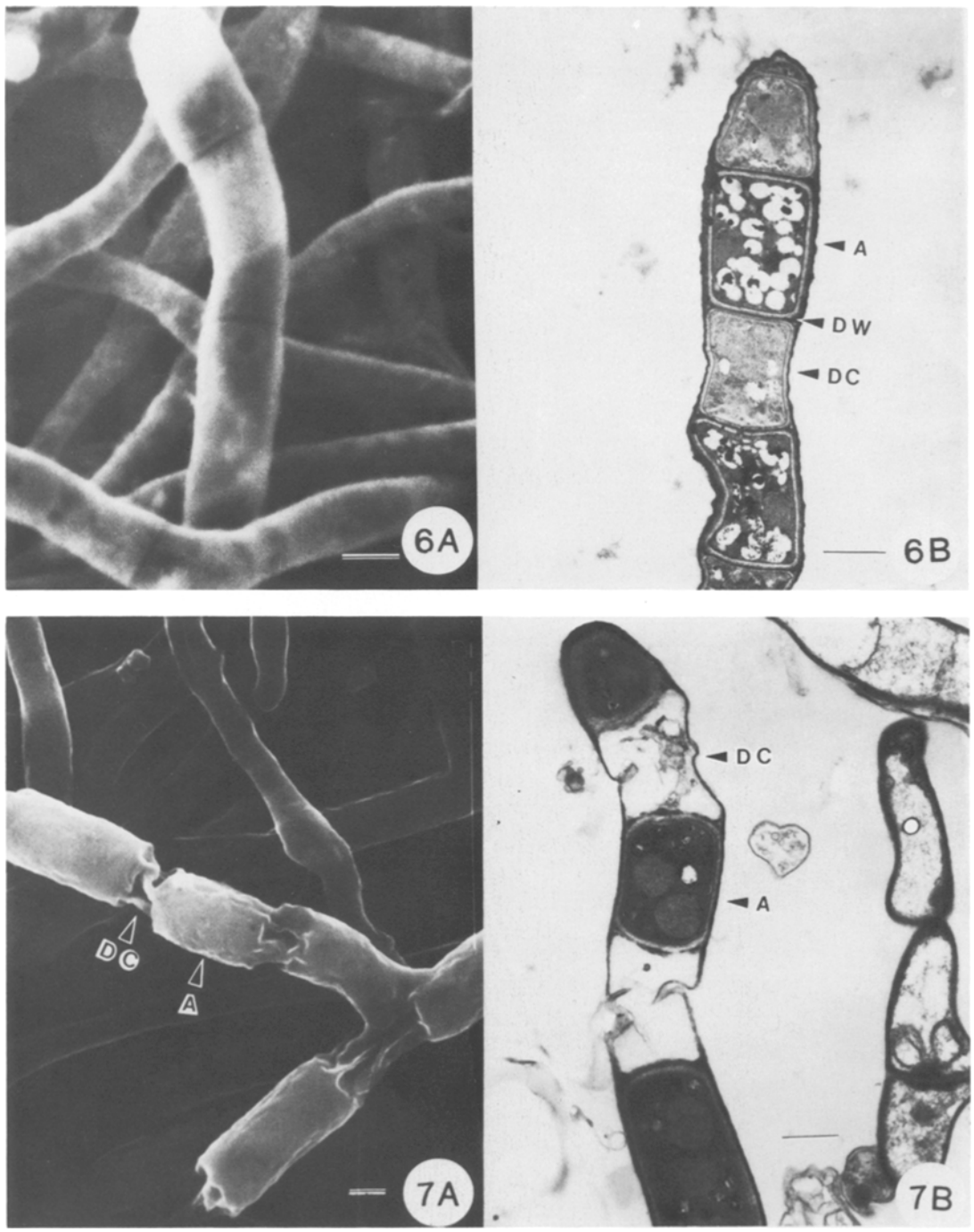


Fig. 6. Maturing fertile hyphae. A, arthroconidium; DC, disintegrating cell; DW, double wall. Bars = 1 μ. (Figure 6B reproduced from 34 by permission.) *Fig. 7.* Mature arthroconidia ready for secession by collapse of disintegrating cell. A, arthroconidium; DC, disintegrating cell. Bars = 1 μ. (Figure 7B reproduced from 34 by permission.)

in an alternating sequence suggests that certain cells are programed to become arthroconidia and others to degenerate.

Parasitic cycle

When mature arthroconidia are cultured under conditions inducing transformation, the earliest event detected is a swelling and rounding of the cell and a change in nuclear cytology. Mature arthroconidia are multinucleate (Fig. 8A), dikaryons being most common. After approximately 8 h under transformation conditions, the cells contain a single nucleus (Fig. 8B) which is 2–3 times larger and stains less intensely than nuclei in arthroconidia. Whether this represents karyogamy or degeneration of all but one nucleus is unknown. The temporal sequence for succeeding stages in the parasitic cycle is strain dependent, varying from 2–5 days for different isolates and not associated with age as a laboratory culture. The round cells are again multinucleate by 24 h (Fig. 9). The OW is irregular and loosely adherent, probably remnants of the arthroconidial OW. During continued incubation, the round cell enlarges, multiple nuclear replications occur (Fig. 10A), and these are synchronous based on the observation that when mitotic figures are present almost all nuclei are involved (Fig. 10B).

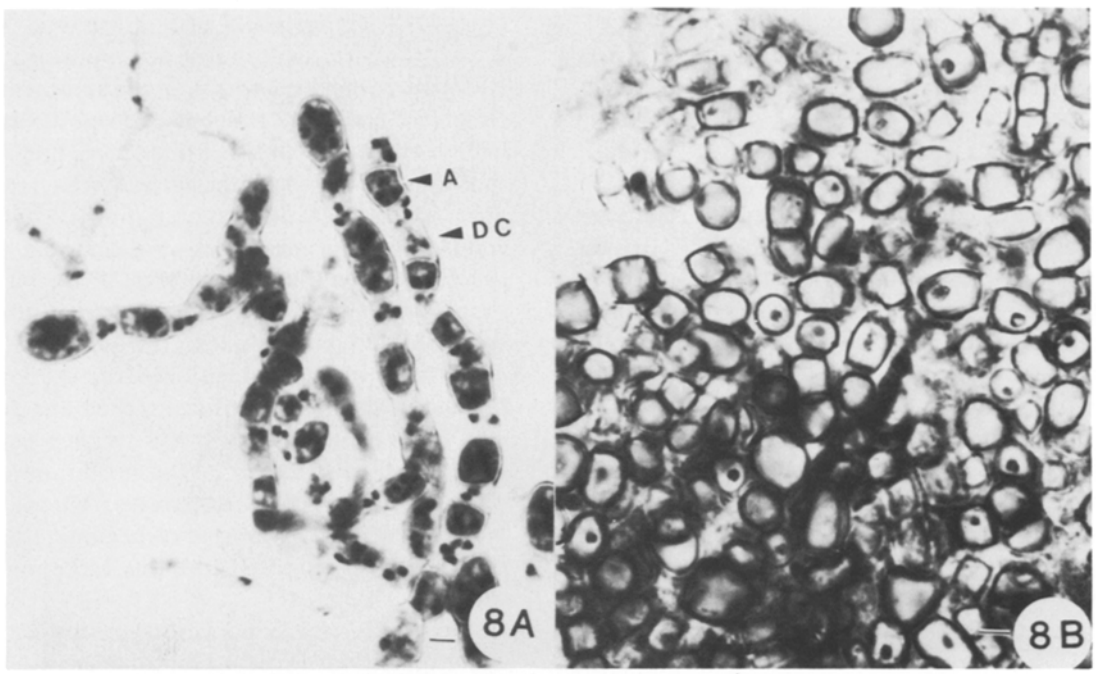
It appears to us that, at some point between O and Q in Figure 1, nuclear replication ceases and segmentation of the protoplasm begins. We do not find any karyokinesis during this period in contrast to earlier stages in which it is frequently apparent and readily demonstrated. Segmentation begins with invagination of the two innermost layers of the multilayered, electron transparent IW (Fig. 11). Since this invagination is composed of wall layers and plasma membrane from both sides of its origin, it contains initially at least four layers with an outer limiting plasma membrane, but the middle two layers, the more electron dense, appear to fuse. The OW surface is irregularly folded. Since the cell surface area is now about 12-fold greater than that of the arthroconidium, it must contain new wall substance rather than only remnants of the original OW of the arthroconidium. Segmentation of the protoplasm is progressive from a primary plane through secondary and successive planes (Fig. 12A), resulting eventually in the formation of endospores (Fig. 12B). Immediately prior to natural release from spherules, endospores are uninucleate and clustered in packets within a hyaline membrane (Fig. 13). Naturally released endospores are still held in clusters by fibrils (Fig. 14A) as they escape through a break in the spherule wall which remains as a shell (Fig. 14B). These fibrils cover the endospores in a meshwork (Fig. 15) and bridge spaces between endospores (Fig. 15B), but they soon break apart. It appears to us that after the spherule wall breaks open, the sac-like hyaline membrane dries to a thread-like structure covering the endospores and then is stretched and twisted into fibrils between endospores as they begin to separate. It is also likely that the membrane derives from the segmentation apparatus. The parasitic cycle initiated with endospores, beginning at stage H in Figure 1, follows the same morphogenetic pattern.

When cells of the parasite cycle are cultured under conditions favoring saprobic growth, germ tube formation depends on the maturation stage of the cell at the time of transfer. Endospores develop only one germ tube (Fig. 16) which is consistent with a uninucleate state. Cells transferred at later stages of maturation usually form multiple germ tubes (Fig. 17) reflecting a multinucleate condition. Septum formation is delayed with endospores but occurs early among cells in later stages of maturation. In both cases, the OW is penetrated and left behind, but the IW is continuous along germ tubes.

Discussion

We have addressed the question of whether host responses might vary with different fungus cell stages throughout the morphogenetic cycle of *C. immitis*. Based on available information, the hypothesis formulated was that these fungus cells at several stages of morphogenesis do induce and elicit different host responses and that these might be due to either qualitative or quantitative differences in macromolecular composition. It seemed reasonable to first examine morphogenesis in considerably more detail than was available currently.

The developmental stages in the saprobic cycle of *C. immitis* are similar to those described for other fungi propagating by alternating enterothallic arthroconidia. Germination of an arthroconidium



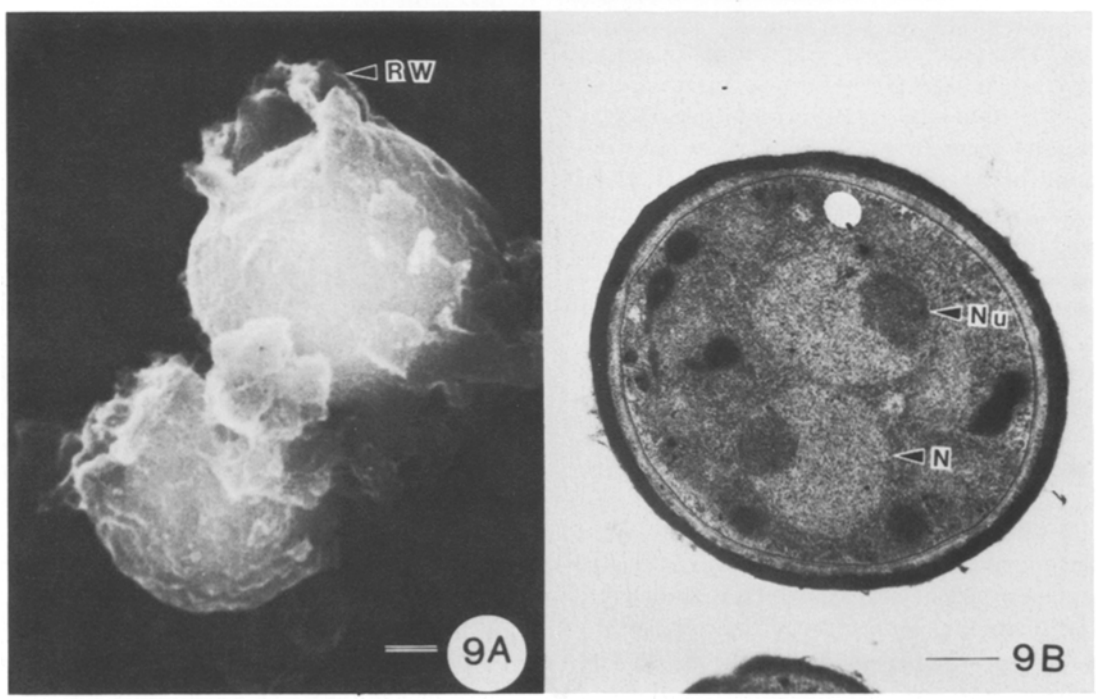


Fig. 8. Multinucleate arthroconidia (A) and rounded uninucleate cells (B) after approximately 8 h incubation under transforming conditions. A, arthroconidium; DC, disintegrating cell. Bars = 1 μ. (Both figures reproduced from 33 by permission.) *Fig. 9.* Round multinucleate cell after 8–24 h (strain dependent) incubation under transforming conditions. N, nucleus; Nu, nucleolus; RW, remnants of outer wall of arthroconidium. Bars = 1 μ.

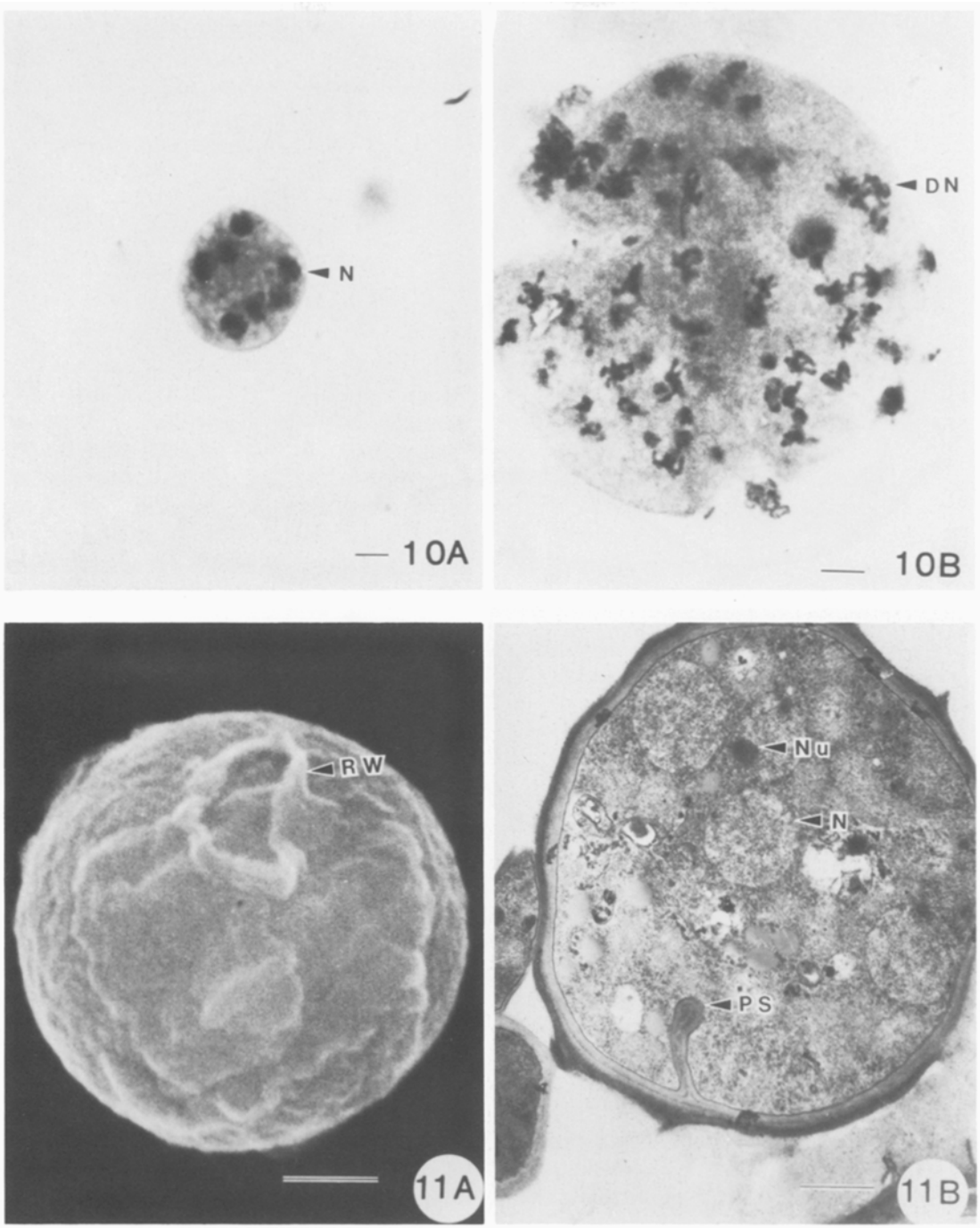


Fig. 10. Nuclear replication during 24–48 h (strain dependent) of maturation in spherule-endospore cycle. Cell walls have been digested to aid staining of nuclei. N, nucleus; DN, dividing nucleus. Bars = 1 μ. (Both figures reproduced from 33 by permission.) *Fig. 11.* Young immature spherule at approximately 48 h (strain dependent) showing initiation of primary segmentation apparatus. N, nucleus; Nu, nucleolus; PS, primary segmentation apparatus; RW, remnants of arthroconidial outer wall. Bars = 1 μ. (Figure 11 B reproduced from 34 by permission.)

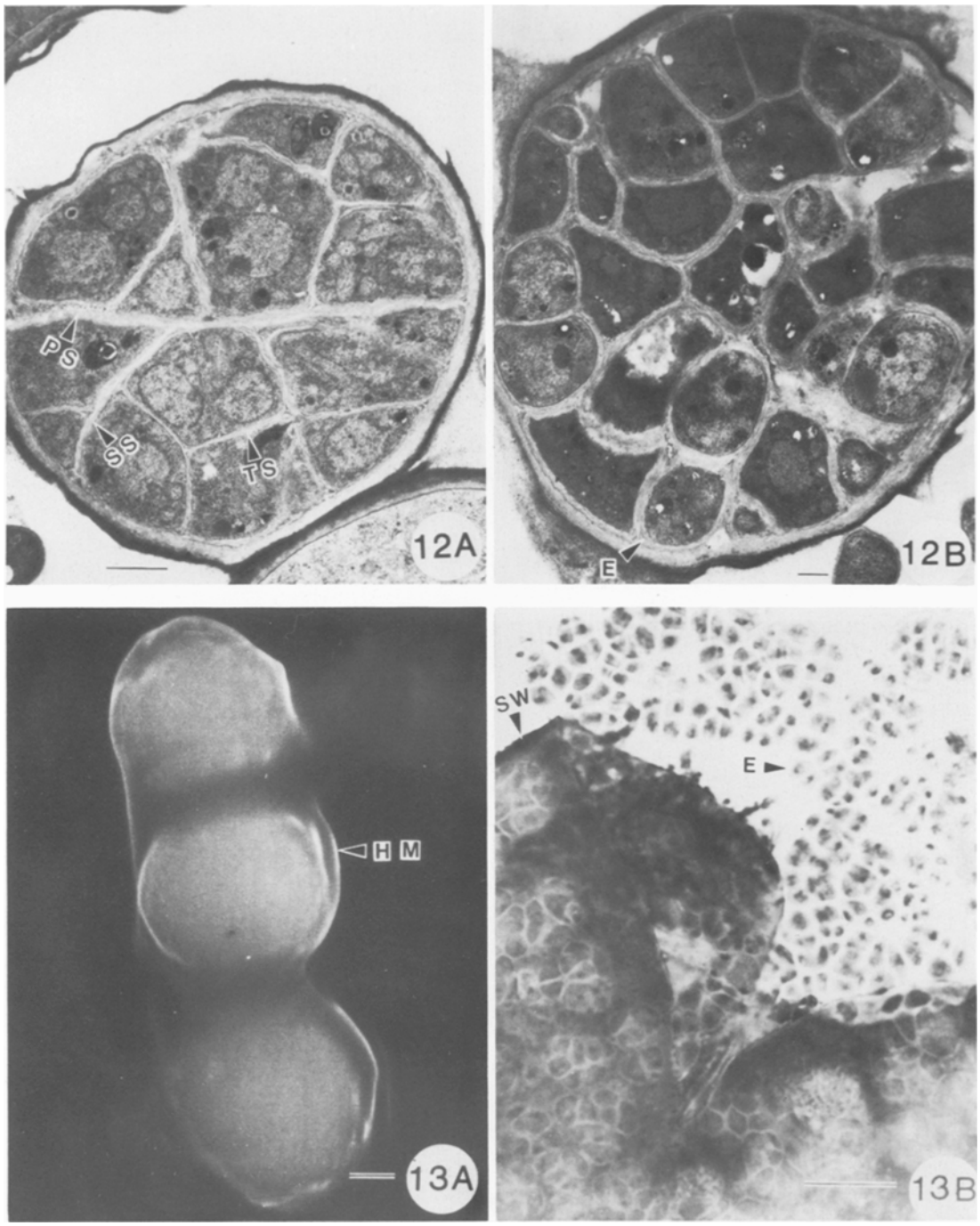


Fig. 12. Formation of endospores by progressive segmentation of protoplasm. E, endospore; PS, SS, and TS are primary, secondary and tertiary segmentation planes, respectively. Bars = 1 μ. (Both figures reproduced from 34 by permission.) *Fig. 13.* Endospores enclosed in a hyaline membrane (A) and uninucleate endospores (B) immediately prior to natural release from spherules. E, uninucleate endospore; HM, hyaline membrane; SW, spherule wall. Bar = 1 μ in A and 10 μ in B. (Figure 13B reproduced from 33 by permission.)

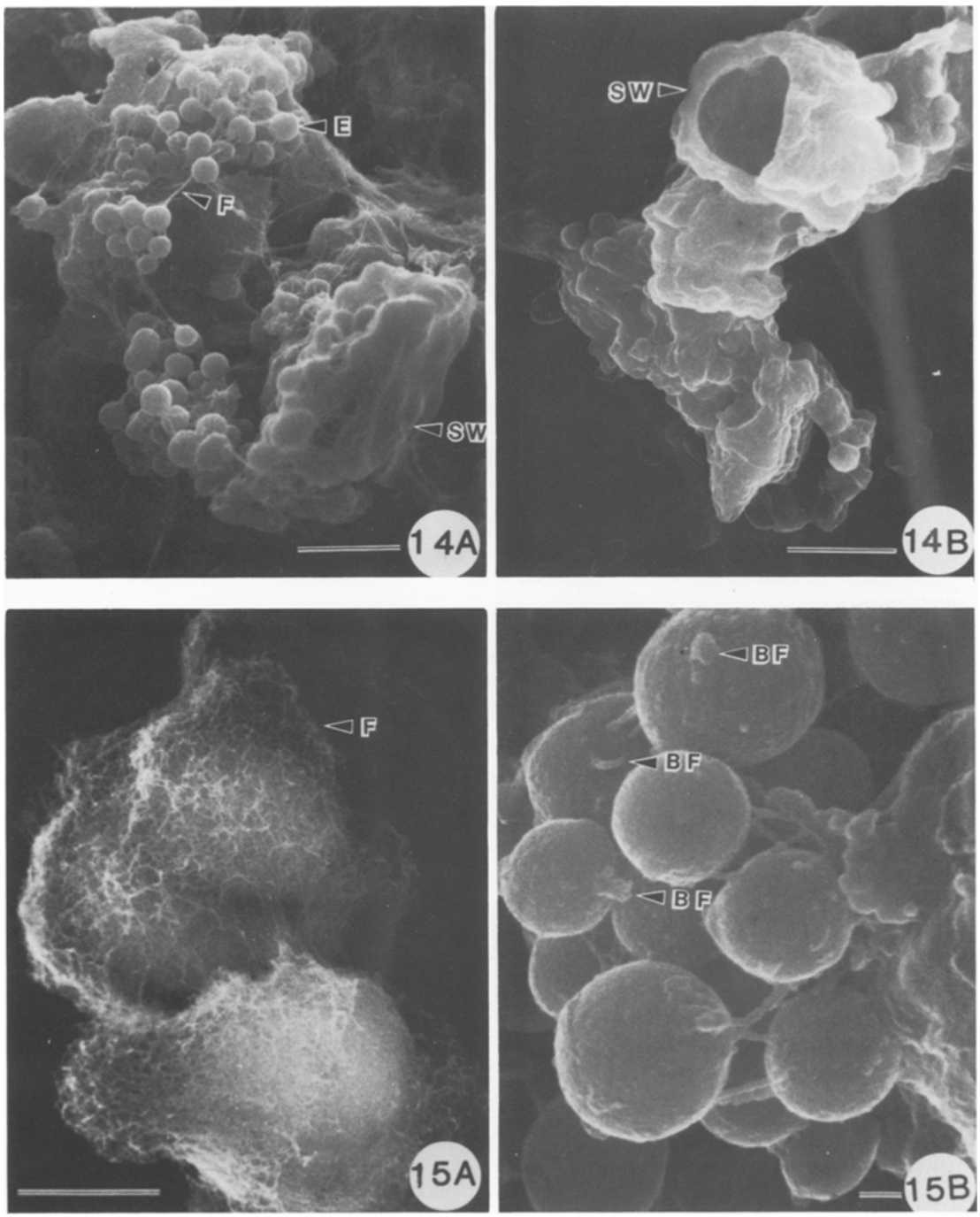


Fig. 14. Naturally released endospores held in clusters by fibrils (A) and leaving spherules as empty shells (B). E, endospore; F, fibril; SW, spherule wall. Bars = 10 μ. *Fig. 15.* Recently released endospores encased in network of fibrils (A) which are stretched and broken as endospores separate (B). BF, broken fibril; F, fibril network. Bars = 1 μ.

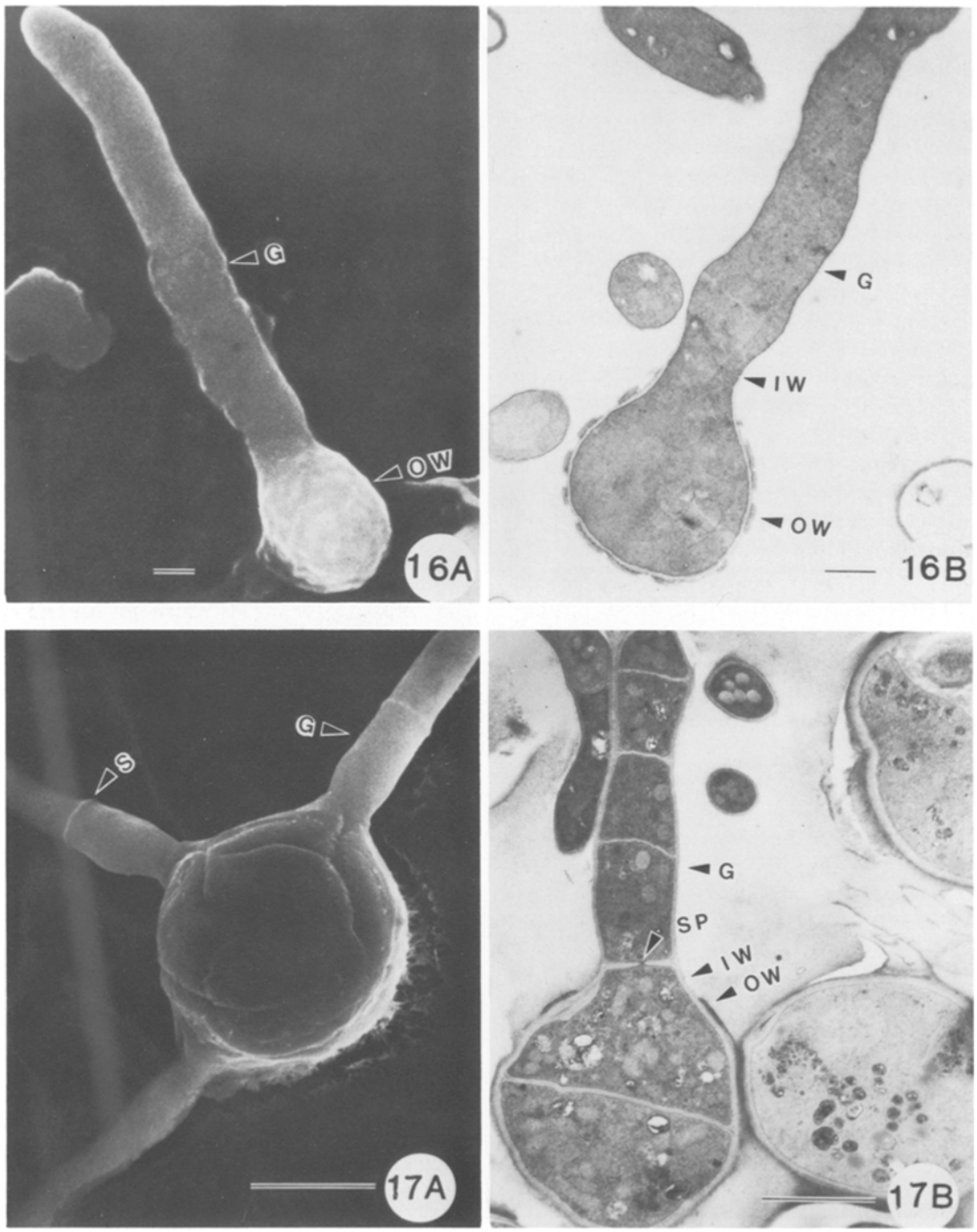


Fig. 16. Germinating endospore after transfer to conditions favoring mycelial growth. G, germ tube; IW, inner wall; OW, outer wall. Bars = 1 μ. *Fig. 17.* Young immature spherule germinating at multiple sites. G, germ tube; IW, inner wall; OW, outer wall; S, septum; SP, septal pore. Bars = 10 μ. (Figure 17B reproduced from 34 by permission.)

leads to mycelial growth composed of septate hyphae with simple septal pores and Woronin bodies (Fig. 4B). The ontogeny of arthroconidia appears identical to that described by Cole and Samson (5) in other similar, but nonpathogenic, species. Certain cells in the fertile hyphae are destined to become arthroconidia and others to degenerate. The characteristic of alternating arthroconidia is not absolute, but it is so predominant that genetic programing must be involved. It is difficult to explain this selection of sequential surviving and degenerating adjacent cells in a single hypha on any other basis. In addition, autolysis of protoplasm and cell wall in the degenerating cell must be exquisitely controlled to insure viability of arthroconidia and survival of the species. Initially, all cells in the fertile hyphae are separated by a double wall (Fig. 6B). Autolysis in cells destined to degenerate proceeds from cytoplasm to plasmalemma to cell wall, but the arthroconidial share of the double wall remains intact (Fig. 7B). Either the thin, electron dense layer in the middle of the double wall is impervious to the autolytic process, or autolysis is terminated by a programmed signal, possibly a feedback from a degradative product. At this point the degenerated cells are essentially empty shells with thin fragile walls subject to collapse (Fig. 7A), fracture (Fig. 7B), and disarticulation of arthroconidia.

Relatively little is known about mechanisms controlling induction and transformation of arthroconidia into the parasitic cycle. The modified Converse medium contains basal salts with dextrose and with ammonium acetate as the sole source of nitrogen. This consistently induces transformation of arthroconidia into the spherule-endospore cycle when cultures are incubated at 40C in an atmosphere of 20% CO_2–80% air. The in vitro requirement for minimal nutrients appears inconsistent with the fact that the spherule-endospore cycle is maintained in the host in the presence, presumably, of rich organic nutrients. Furthermore, several investigators have produced this transformation in fertile chick eggs (4, 24) and in tissue cultures, using HeLa cells (2) or polymorphonuclear leukocytes (1). Two possibilities merit consideration. Either the inductive and directive molecular mechanisms in the in vitro system differ from those in host systems, or the species is programmed to respond to unfavorable environments by transformation into the spherule-endospore cycle with either minimal nutrient supply in vitro or host cell response in vivo triggering the transformation. The latter hypothesis appears more plausible. We have matched morphological stages in experimentally infected mice with those seen in the in vitro system (33). In addition, figures and descriptions by Donnelly and Yunis (8) of spherules and endospores demonstrated by TEM in human necropsy material also match in vitro parasitic forms. Endospores occurred in clusters, and their outer wall appeared 'granular and fibrillar' suggesting 'that they consisted of overlapped, nearly thatched layers of fibrils' which resembled the material that 'lay between closely apposed sporangiospores' within spherules. These descriptions resemble the meshwork of fibrils covering endospores which, in our opinion also, are derived from the segmentation apparatus. Thus, the observable morphological events appear identical in both in vitro and in vivo systems, but the genetic and molecular mechanisms which govern and execute the transformation remain unknown.

The first spherule-endospore cycle is initiated by an arthroconidium, but succeeding cycles derive from endospores, and there may be a fundamental difference in nuclear ontogeny of each one. The mature arthroconidium is multinucleate (Fig. 8A), but becomes uninucleate (Fig. 8B) early in the transformation period. Either karyogamy has occurred, or all nuclei but one have degenerated. In contrast, present evidence indicates that uninucleate endospores derive from progressive segmentation of the multinucleate protoplasm in the maturing spherule. We cannot exclude the possibility of karyogamy at some point during the maturation period, but careful study of successive preparations shows only a period of rapid nuclear replication (Fig. 10) followed by a period in which all nuclei appear in interphase while segmentation proceeds (Fig. 11, 12). Thus, although each spherule-endospore cycle begins with an uninucleate cell, the first cycle originates from a multinucleate cell in which all but one nucleus have disappeared while succeeding cycles originate from primary uninucleate cells.

Several distinct stages in the spherule-endospore cycle appear programed. There must be molecular signals which initiate rapid and synchronous nuclear replication, and then cessation of the nuclear replication and induction of the segmenta-

tion apparatus. In addition, we do not know whether release of endospores is entirely mechanical and caused by increasing internal pressure, or if there is a control mechanism functioning to weaken a localized area of the spherule wall and timed to insure maturation of endospores before release. There is much to be learned about molecular mechanisms and genetic programing which govern morphogenesis in each cycle and transition from one to the other.

The immunology of coccidioidomycosis has been examined with extracts containing multiple antigens (15, 16). These have served remarkably well as aids for diagnosis and prognosis (9, 29–31), but additional knowledge of the host-parasite interaction in this mycosis, especially as a model of infection in which CMI is determinative, will require identification of significant antigens and correlation with morphogenesis in the parasitic cycle. Growth and differentiation should be associated with both qualitative and quantitative changes in macromolecules and, in the case of a pathogen, these might induce or elicit different host responses.

The available information about immunological responses induced or elicited by cells or cell walls from stages in the life cycle of *C. immitis* indicates that differences exist. Although disseminated coccidioidomycosis is referred to generally as a granulomatous disease, the overall histopathology is one of mixed suppurative and granulomatous responses (13, 14), and the former is associated primarily with released endospores, and the latter with spherule development (11, 12). This evidence from the host cell response suggests that endospores differ from cells in later stages of maturation. Tarbet and Breslau (35), using histochemistry, found no phospholipid in walls of endospores but increasing amounts as cells matured to spherules. In contrast, the outer layer of endospores was rich in mucopolysaccharide which was inconstant in spherules and only in vacuoles when present. They suggested that these differences might be related to the primarily polymorphonuclear cell response to endospores and mononuclear cell response to spherules. Collins and Pappagianis (6) reported that cell walls of mature endosporulating spherules were markedly reduced in thickness by both chitinase and lysozyme, but walls of immature spherules were not affected by the enzymes. In addition, 19% of washed spherule wall was solubilized as glucose by chitinase, which contained $\beta(1 \rightarrow 3)$-glucanase, but none was obtained from endospore walls. Antigenic differences among fungus cells were found with respect to complement fixation (CF) and tube precipitin (TP) reactivity (7). When washed walls from immature (6–12 μm) and mature (30–35 μm) spherules were incubated with lysozyme, chitinase, or buffer without enzyme, CF and TP reactive antigens were detectable in all supernatants from mature spherule walls, but walls from immature spherules yielded only TP antigen in the supernatant from chitinase treatment. Since chloroform was used in all suspensions to kill cells and prevent contamination, and probably affected cell membrane permeability, the cellular location of these antigens needs confirmation. It should be noted, however, that cell walls were washed extensively and adsorption of cytoplasmic antigens was probably minimal. Levine *et al.* (21) demonstrated that vaccination with formalin killed cells induced resistance in mice to respiratory challenge with arthroconidia, and that vaccine efficacy was best with spherules, less with endospores, and least with arthroconidia. Since comparable protection was achieved with isolated spherule walls (19), Levine *et al.* (22) suggested that 'the morphogenetic development of the endospore was accompanied by antigenic changes in its wall and these accounted, at least in part, for the spherule's immunogenicity.' Thus, cells of *C. immitis* during stages of maturation exhibit certain differences in macromolecular composition and some of these are antigen related.

Several aspects of the life cycle of *C. immitis* deserve attention in relation to pathogenesis. Smith (32) has stated that mechanisms by which surface components can interfere with host defenses include '... hindering contact with phagocytes; ... preventing ingestion by phagocytes; ... interfering with intracellular killing by phagocytes ...'. The OW of the arthroconidium is incomplete since it is broken at secession from the parent fertile hyphae. Presumably, the attachment to the IW is weak because the OW can be shed at germination (Fig. 3A). During earliest infection, the infecting arthroconidium is engaged by host phagocytic cells (27), and it is possible that a loosely adherent OW might present a problem either for phagocytosis or for killing mechanisms within a phagosome. Whether the arthroconidial OW can function as an 'aggres-

sin' (32) remains to be determined. Endospores and spherules also differ in composition of cell walls and immunogenicity as noted above. In addition, the wall of newly released endospores is derived from the segmentation apparatus, but the cell acquires an electron dense OW as maturation proceeds. These differences could affect phagocytosis and survival in the host.

Smith (32) also states, 'The more rapid the rate of multiplication, the more likely is infection to be established despite the activity of the host defense mechanisms.' Maturation during the parasitic cycle must be accompanied by a vigorous anabolic activity because there is rapid karyokinesis and increase in cell mass with the net result that a single cell has produced several hundred progeny. This occurs encased within a wall which increases in thickness during the maturation period and may be protective with respect to killing after phagocytosis. Huntington (13) noted that this fungus has a 'decided propensity for establishing itself within epithelioid and giant cells' despite its large size. It is likely that phagocytosis occurred at an early stage but failed to prevent growth of the fungus.

Thus, the available evidence indicates that cells from stages in the morphogenetic cycle of *C. immitis* differ with respect to phagocytosis mechanisms, potential for inducing a protective response to challenge infection, and for eliciting CMI or serological responses. Additional information is needed to determine whether these differences are qualitative or quantitative, and whether cellular location of macromolecules at different morphogenetic stages determines presentation of antigens to, and response from, host cells.

Acknowledgements

This work was supported by the General Medical Research Service of the Veterans Administration.

Part of this work was presented in the seminar, Fungal dimorphism: Morphological and biochemical aspect, at the 81st Annual Meeting of the American Society for Microbiology, 1981.

References

1. Baker, O. & Braude, A. I., 1956. A study of stimuli leading to the production of spherules in coccidioidomycosis. J. Lab. Clin. Med. 47: 169–181.
2. Bartels, P. A., Wagoner, N. & Larsh, H. W., 1968. Conversion of Coccidioides immitis in tissue culture. Mycopathol. Mycol. Applic. 35: 37–41.
3. Breslau, A. M., Hensley, T. J. & Erickson, J. O., 1961. Electron microscopy of cultured spherules of Coccidioides immitis. J. Biophys. Biochem. Cytol. 9: 627–637.
4. Brueck, J. W. & Buddingh, G. J., 1951. Propagation of pathogenic fungi in the yolk sac of embryonated eggs. Proc. Soc. Exper. Biol. Med. 76: 332–335.
5. Cole, G. T. & Samson, R. A., 1979. Patterns of development in conidial fungi. Fearon Pitman Publ. Inc., Belmont, Calif.
6. Collins, M. S. & Pappagianis, D., 1973. Effects of lysozyme and chitinase on the spherules of Coccidioides immitis in vitro. Infect. Immun. 7: 817–822.
7. Collins, M. S., Pappagianis, D. & Yee, J., 1977. Enzymatic solubilization of precipitin and complement fixing antigen from endospores, spherules, and spherule fraction of Coccidioides immitis, p. 429–444. In: L. Ajello (ed.), Coccidioidomycosis: Current clinical and diagnostic status. Symposia Specialists, Miami, Fla.
8. Donnelly, W. H. & Yunis, E. J., 1974. The ultrastructure of Coccidioides immitis. Study of a human infection. Arch. Pathol. 94: 227–232.
9. Drutz, D. J. & Catanzaro, A., 1978. Coccidioidomycosis. Am. Rev. Resp. Dis. 117: 559–585, 727–771.
10. Emmons, C. W., 1967. Fungi which resemble Coccidioides immitis, p. 333–337. In: L. Ajello (ed.), Coccidioidomycosis: Proceedings second coccidioidomycosis symposium. Univ. Ariz. Press, Tucson, Ariz.
11. Fiese, M. J., 1958. Coccidioidomycosis. Charles C. Thomas, Publ., Springfield, Ill.
12. Forbes, W. D. & Bestebreurtje, A. M., 1946. Coccidioidomycosis, a study of 95 cases of the disseminated type with special reference to the pathogenesis of the disease. Milit. Surg. 59: 653–719.
13. Huntington, R. W., 1957. Diagnostic and biologic implications of the histopathology of coccidioidomycosis, p. 36–46. In: Proceedings of symposium on coccidioidomycosis. U. S. Publ. Health. Serv. Public. No. 575.
14. Huntington, R. W., 1980. Pathology of coccidioidomycosis, p. 113–132. In: D. A. Stevens (ed.), Coccidioidomycosis: A text. Plenum Med. Book Co., New York, N.Y.
15. Huppert, M., Adler, J. P., Rice, E. H. & Sun, S. H., 1979. Common antigens among systemic disease fungi analyzed by two-dimensional immunoelectrophoresis. Infect. Immun. 23: 479–485.
16. Huppert, M., Kinsey, N., Sun, S. H., Vukovich, K. R. & Rice, E. H., 1978. Antigenic analysis of coccidioidin and spherulin determined by two-dimensional immunoelectrophoresis. Infect. Immun. 20: 541–551.
17. Huppert, M., Sun, S. H. & Bailey, J. W., 1967. Natural variability in Coccidioides immitis, p. 323–328. In: L. Ajello (ed.), Coccidioidomycosis: Proceedings second coccidioidomycosis symposium. Univ. Ariz. Press, Tucson, Ariz.
18. Kendrick, B. (ed.), 1971. Taxonomy of fungi imperfecti. Univ. Toronto Press, Toronto, Queb.
19. Kong, Y.-C. M., Levine, H. B. & Smith, C. E., 1963. Immunogenic properties of nondisrupted and disrupted spherules of Coccidioides immitis in mice. Sabouraudia 2: 131–142.

20. Kwon-Chung, K. J., 1969. Coccidioides immitis: Cytological study on the formation of arthrospores. Canad. J. Genet. Cytol. 11: 43–53.
21. Levine, H. B., Cobb, J. M. & Smith, C. E., 1960. Immunity to coccidioidomycosis induced in mice by purified spherule, arthrospore, and mycelial vaccines. Trans. N. Y. Acad. Sci. 22: 436–449.
22. Levine, H. B., Pappagianis, D. & Cobb, J. M., 1970. Development of vaccines for coccidioidomycosis. Mycopathol. Mycol. Applic. 41: 177–185.
23. Maddy, K. T., 1965. Observations on Coccidioides immitis found growing naturally in soil. Ariz. Med. 22: 281–288.
24. Moore, M., 1941. The chorioallantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi. Am. J. Pathol. 17: 103–120.
25. O'Hern, E. M. & Henry, B. S., 1956. A cytological study of Coccidioides immitis by electron microscopy. J. Bacteriol. 72: 632–645.
26. O'Hern, E. M. & Henry, B. S., 1957. An electron microscope study of Coccidioides immitis, p. 191–197. In: Proceedings of symposium on coccidioidomycosis. U. S. Publ. Health Serv. Public. No. 575.
27. Savage, D. C. & Madin, S. H., 1968. Cellular responses in lungs of immunized mice to intranasal infection with Coccidioides immitis. Sabouraudia 6: 94–102.
28. Sigler, L. & Carmichael, J. W., 1976. Taxonomy of Malbranchea and some other hyphomycetes with arthroconidia. Mycotaxon 4: 349–488.
29. Smith, C. E., Saito, M. T., Beard, R. R., Kepp, R. M., Clark, R. W. & Eddie, B. U., 1950. Serological tests in the diagnosis and prognosis of coccidioidomycosis. Am. J. Hyg. 52: 1–21.
30. Smith, C. E., Saito, M. T. & Simons, S. A., 1956. Pattern of 39,500 serologic tests in coccidioidomycosis. J. Am. Med. Assoc. 160: 546–552.
31. Smith, C. E., Whiting, E. G., Baker, E. E., Rosenberger, H. G., Beard, R. R. & Saito, M. T., 1948. The use of coccidioidin. Am. Rev. Tuberc. 57: 330–360.
32. Smith, H., 1977. Microbial surfaces in relation to pathogenicity. Bacteriol. Rev. 41: 475–500.
33. Sun, S. H. & Huppert, M., 1976. A cytological study of morphogenesis in Coccidioides immitis. Sabouraudia 14: 185–198.
34. Sun, S. H., Sekhon, S. S. & Huppert, M., 1979. Electron microscopic studies of saprobic and parasitic forms of Coccidioides immitis. Sabouraudia 17: 265–273.
35. Tarbet, J. E. & Breslau, A. M., 1953. Histochemical investigation of the spherule of Coccidioides immitis in relation to host reaction. J. Infect. Dis. 92: 183–190.