NOTES

EFFECT OF SURFACE ACTIVE AGENTS ON ENDOSPORULATION OF *COCCIDIOIDES IMMITIS* IN A CHEMICALLY DEFINED MEDIUM

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The chief obstacles to the study of the parasitic or *in vivo* form (spherule) of *Coccidioides immitis* grown in synthetic media have been: (1) separating spherules from hyphae, (2) obtaining rupture of the majority of the spherules, and (3) failure of the endospores to separate from the spherule matrix. Growth of spherules in a chemically defined liquid medium composed of glucose, ammonium acetate, and inorganic salts has been reported (Converse, Proc. Soc. Exptl. Biol. Med., **90,** 709–711, 1955 and J. Bacteriol., **72,** 784–792, 1956). The present report is concerned with the production of essentially pure (hyphae-free), uniparticulate cultures of the parasitic growth form (liberated endospores) in a defined medium, and the ability to perpetuate the parasitic reproductive cycle (endospore → spherule → endospore) through several serial transfers.

Representative types of anionic, cationic, and nonionic surface active agents were investigated to determine their stimulatory effect on sphérulation, their effect on the surface of the spherule wall, and their ability to disperse liberated endospores throughout the medium. Each compound was added to the basal medium at several concentrations. Fifty-ml volumes of the media were inoculated with 1×10^7 viable particles (arthrospores and hyphal fragments from a culture grown in liquid synthetic medium) of *C. immitis* strain M11 and incubated with shaking for 72 hr at 34 C.

TABLE 1

Effect of Tamol "N" on growth and spherulation of Coccidioides immitis

Tamol "N"†	Growth‡	Spherule‡ Yield	Viable Plate Count*($\times 10^6$)		Morphology
			Before filtration	After filtration	
1.0	5	8	25	22	98% Free endospores. Good dispersion.
0.5	5	8	26	30	
0.1	4–5	8	28	31	
0.05	4–5	8	33	39	
0.01	4	7–8	19	11	90% Free endospores. Good dispersion. Few arthrospores present.
0.005	4	6			Few free endospores and arthrospores present. Many hyphae.
0.001	3	1			Many arthrospores and hyphae. Poor dispersion.
0.0 (control)	4	1–4	11	4	

* Mean values of 3 to 7 cultures.

† Added to basal spherule medium (Converse, J. Bacteriol. **72,** 784–792, 1956).

‡ Visual estimations based on arbitrary scale of 0–8.

[1] With the technical assistance of HM1 Arthur R. Besemer, U.S.N.

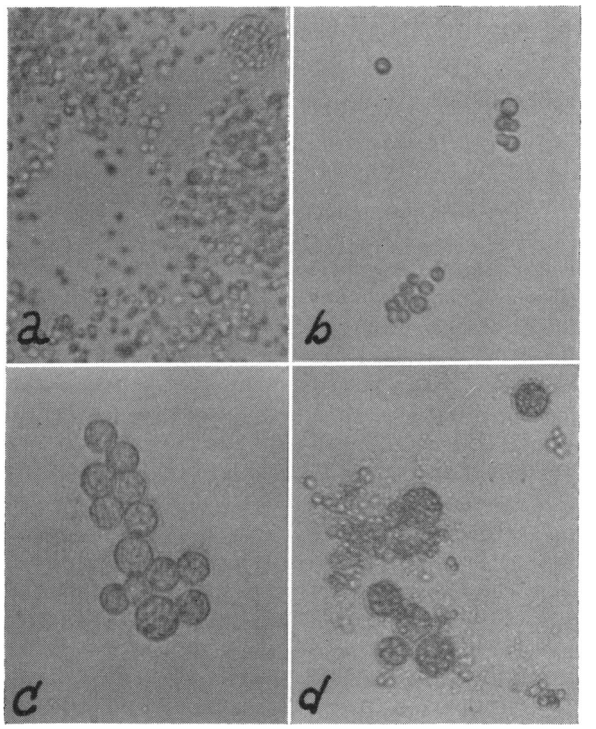

Figure 1. Complete cycle of the parasitic (*in vivo*) form of reproduction in synthetic medium containing 0.05 per cent Tamol "N" (323 ×): *a.* Endospore inoculum (grown in the presence of 0.05 per cent Tamol "N". *b.* Endospore development 24 hr after transfer. *c.* Endospore development 48 hr after transfer (note formation of cross walls). *d.* Endospore development into mature spherules 72 hr after transfer (rupture of some of the spherules has taken place, releasing the next generation of endospores).

Tamol "N", an anionic surface active agent (sodium salt of a condensed aryl sulfonic acid; Rohm and Haas Co., Philadelphia, Pa.), was more effective than any of the other compounds tested. Table 1 shows its action as (1) a growth stimulant in concentrations of 0.05 to 1.0 per cent, (2) a spherulation stimulant (98 per cent spherules) in concentrations of 0.005 to 1.0 per cent, and (3) a dispersing agent in concentrations of 0.01 to 1.0 per cent. Moreover, its action on the spherule walls resulted in rupture of 90 to 95 per cent of the spherules present in the cultures, with subsequent dispersion of free endospores throughout the medium (figure 1 *a*). Filtration of the culture through 6 layers of surgical gauze removed the trace amounts of hyphae present without significantly altering the total endospore yield as determined by viable plate counts before and after filtration. The resulting uniparticulate suspensions contained 1 to 7 × 10^7 endospores per ml.

The parasitic or *in vivo* reproductive cycle was maintained through three serial transfers (at 72-hr intervals) in basal spherule medium supplemented with 0.05 or 0.1 per cent Tamol "N." Figure 1 shows the development (at 24-hr intervals) of endospores into spherules in one complete reproductive cycle.