10060 P

Spherule Formation and Endosporulation of the Fungus Coccidioides *in vitro*.*

ARTHUR R. LACK. (Introduced by Ernest C. Dickson.)

From the Department of Public Health and Preventive Medicine, Stanford University School of Medicine, San Francisco.

Since the causative agent of coccidioidal granuloma (*Coccidioides immitis* Stiles) was demonstrated to be a fungus and not a protozoon by Ophüls and Moffitt,[1] the reason for the formation of spherules in the animal body has evaded explanation. MacNeal and Taylor[2] were the first to attempt to obtain growth of spherules outside of the animal body. They inoculated pus containing spherules into tubes of ascitic fluid and gelatinized horse-serum to which sterile tissue had been added and cultured them anaërobically. They observed that parasitic forms, *i. e.*, spherules, continue to multiply for a time by endogenous formation. Takahashi[3] repeated the experiments of MacNeal and Taylor, but was unable to observe the formation of endosporulating spherules. Ciferri and Redaelli,[4] however, were able to confirm the continued formation of endosporulating spherules from pus containing non-endosporulating spherules. On the other hand, their anaërobic tubes inoculated with fungus from normal cultures showed nothing interesting.

In an effort to find the prerequisite conditions for spherule-formation in the case of coccidioides, a series of semi-anaërobic experiments was performed.

Procedure: A semi-anaërobic constricted tube of the Hall[5] type was used. A series of tubes was filled well above the point of constriction with filtered glucose-broth having a pH of 7.2 and heated to 100°C for 15 min. When cooled to room temperature, they were ready for inoculation. The inoculum was prepared by extracting the albumin from a fresh egg and suspending in it a preparation of chlamydospores from an 8 weeks' old fungus culture (from the Stanford Fungus Series, strain No. 46). This inoculum was

* This study is being aided by the Rosenberg Foundation, San Francisco.

1 Ophüls, W., and Moffitt, H. C., *Phil. Med. J.*, 1900, **5**, 1471.

2 MacNeal, W. J., and Taylor, R. M., *J. Med. Research*, 1914, **30**, 261.

3 Takahashi, S., *Arch. f. Dermat. u. Syph.*, 1933, **168**, 597.

4 Ciferri, R., and Redaelli, P., *Soc. internaz. di microbiol., Boll. d. sez. ital.*, 1934, **6**, 141.

5 Hall, I. C., *J. Inf. Dis.*, 1921, **29**, 317.

pipetted into the bottom of the constricted tubes and immediately a sterile marble large enough to close off the constricted neck was dropped in place. By gentle warming partial coagulation of the egg albumin within the lower chamber was obtained, the heat being insufficient either to kill the organism or completely coagulate the egg albumin. Four control tubes were prepared: one as described above but uncoagulated by heat, one containing the inoculum with no marble, one with fresh egg albumin and one with the suspension of chlamydospores without egg albumin. The tubes were then incubated at 37°C. Samples were taken every 24 hr and examined by coverslip and stained smear preparations.

Results: At 24 hr, the experimental tubes show a slight enlargement of the chlamydospores within the egg albumin. A rounding-out of previously oval shaped chlamydospores takes place. There is no development of mycelial buds (hyphæ) from either the chlamydospores or the broken mycelial fragments contained in the original chlamydospore suspension. The controls show no changes except for beginning hyphæ formation in the tube containing the fungus without the egg albumin.

By 48 hr, the growth or enlargement of the chlamydospores in the experimental tubes continues with the formation of now small, spherule-like bodies, round in contour and possessing a distinct, greenish capsule. Round bodies are seen which are 20-30μ in diam, are finely granular in structure and possess a greenish, granular capsule. These may be designated the "granular" type of spherule. In these same cultures are found larger round organisms (30-40μ in diam), granular in structure and possessing a thick yellow-green capsule, from which peripherally radiate numerous large spicules. These spicules are quite distinct in structure, being broad at their base and quickly narrowing to a very sharp point; they are analogous to the prickle-forms described by Rixford and Gilchrist.[6] Again the controls show no changes except for growth of mycelium in the tube containing the fungus alone.

After 7 days, coverslip-examinations of portions of the egg albumin of the experimental tubes show many greenish, granular spherules, some of which have the sharp, broad spicules. There are a few clear (vacuolated) spherules possessing wide, refractile, double-contoured capsules which are yellowish green in color. A large spherule of approximately 50μ in diam, with a greenish capsule of medium width, is seen. Within this organism are round or

[6] Rixford, E., and Gilchrist, T. C., *Johns Hopkins Hosp. Reports*, 1896, **1**, 209.

irregularly shaped bodies about 6-10μ in diam. These are endobodies or endospores. The control tubes show no spherule-development; mycelial growth is obtained in the tube with no marble and in the upper portion of the tube with coccidioides alone.

The formation of endospores in the spherules obtained in this series establishes the identity of the spherule-form. It also indicates the maturity of the organism and renders the *in vitro* development of the fungus under certain conditions comparable to the *in vivo* development of the same fungus.

Summary: The parasitic cycle of the fungus coccidioides was reproduced *in vitro*. Spherule-formation with endosporulation was experimentally produced under certain conditions, including partial anaërobiosis and the presence of partially coagulated egg albumin. An interesting type of spherule-formation (granular spherule) and a distinct spicule formation are described.

10061

Influence of Ketene on the Potency of Antipneumococcus Serum.*†

JOSEPH T. TAMURA AND M. JOHN BOYD. (Introduced by S. Tashiro.)

From the Departments of Bacteriology and Biochemistry, College of Medicine, University of Cincinnati.

Recently we[1] showed that the treatment of anti-Brucella horse serum with ketene for 35 min or more prevents anaphylactic shock in animals sensitized to the original antiserum. Furthermore, such a serum still retains the major part of its agglutinating antibody. The purpose of this paper is to record the influence of ketene on the protective power of antipneumococcus serum.

Type I antipneumococcus horse serum, containing 200 units per cc (1642-1, Eli Lilly and Co.) was tested. To 10 cc of the serum to be ketenized, 0.6 g of $NaHCO_3$ was added to buffer the acetic

* This research was aided in part by a grant from the Craig Yeiser Fund, College of Medicine, University of Cincinnati.

† The authors wish to acknowledge the courtesy of Dr. Mary B. Kirkbride of the New York State Department of Health, and Dr. H. M. Powell of the Eli Lilly and Company.

[1] Boyd, M. J., and Tamura, J. T., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 184.