ampoules or lyophilized. The results by this procedure are shown in Table II.

As a final check on the virus inactivation by this procedure, a fresh lot of the soluble antigen was prepared in the usual manner. One aliquot of it was deliberately contaminated with PR8 virus by adding to it the pellet containing the virus which was obtained by centrifuging an equivalent volume of infected allantoic fluid. The soluble antigen had a hemagglutination titer of less than 20 units per ml and the ID_{50} was $10^{-6.5}$ whereas the antigen plus virus had a hemagglutination titer of 2560 units per ml and the ID_{50} was $10^{-8.7}$. The samples were then treated with 0.1% and 0.15% formaldehyde, both with and without subsequent neutralization. Infectivity tests showed all samples to be non-infective and the neutralized samples had lost no antigenicity before or after lyophilization.

At the time of writing, samples of antigens treated with hydrogen peroxide or formaldehyde plus neutralization have been stored for over 2 months in sealed ampoules and in the dried condition at 4°C and have lost none of their antigenicity nor have they become anti-complementary.

Discussion. It can be seen from Table I that (1) when the soluble influenza A (PR8) antigen contained 3% hydrogen peroxide, the infectivity was destroyed only under conditions which caused a large loss of the antigenicity (2) treatment with 2% peroxide at 22°C for 2 hours, followed by lyophilization, produced a non-infective antigen with little or no loss of antigenicity (3) treatment with 1% peroxide at 37°C for 1 hour followed by an increase to 2% peroxide at 22°C for 1 hour yielded an antigen which was non-infective after lyophilization and which had lost little or none of its antigenicity. In the 3 experiments done, this antigen was also non-infective prior to lyophilization.

From Table II it is evident that when the concentration of formaldehyde in the antigen is sufficient to destroy the infectivity, lyophilization destroys the antigenicity. However, when the excess formaldehyde is neutralized with ammonia prior to lyophilization, the antigenicity remains intact. The fact that the reaction temperature can be increased to 37°C for 2 hours and the ammonia concentration to pH 10 without loss of the antigenicity indicates that the antigen is quite stable to this method of treatment. Normal membrane antigen treated by this procedure gave no significant nonspecific antigen titer in the complement-fixation test.

Since the antigenicity is more stable to variations in temperature and reagent concentration with the formaldehyde treatment, it is considered to be the better method.

Summary. Two methods for the preparation of a stable non-infective soluble influenza A antigen have been developed. One is based on treatment with 2% hydrogen peroxide, the other on treatment with 0.1% formaldehyde followed by its neutralization prior to storage or lyophilization.

Received January 13, 1951. P.S.E.B.M., 1951, v76.

In Vitro Cultivation of the Parasitic Phase of *Coccidioides immitis.* (18481)

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To date only partially successful results have been obtained in attempts to culture the parasitic phase of *Coccidioides immitis* which exists as a spherical endospore-producing organ (commonly known as 'spherule') *in vivo* and as a fluffy white mold *in vitro.*

* The author wishes to thank Prof. W. H. Weston for aid and counsel during the course of this work; Miss Betty Insley and Dr. G. Morel for tubes of coconut milk agar medium; Dr. E. E. Baker for suggestions concerning the research, and Dr. N. F. Conant for supplying 5 strains of *C. immitis.*

Continued multiplication of the spherules for as long as 3 weeks was noted by MacNeal and Taylor(1) when pus containing the organisms was added to ascitic fluid or to gelatinized horse serum containing sterile kidney slices. The *in vitro* transformation of culture-grown chlamydospores into spherules was noted first by Lack(2) who used Hall tubes containing glucose broth and partially coagulated egg albumin in which chlamydospores were suspended. Baker and Mrak(3) reported 2 out of 16 strains of *C. immitis* to produce *in vitro* structures closely resembling spherules. These structures, called "culture spherules," were produced under aerobic conditions at room temperature and 37°C and were found to develop on a variety of media.

The present paper reports the *in vitro* formation of spherules, or sporangia as they are more properly termed, by all of 7 representative strains of *C. immitis* tested: strain VA 10 was isolated by Mrs. Lily Anisman, and strain VA 7 was isolated by the author at the Veterans Adm. Hosp., Sawtelle, Los Angeles, Calif. Strains 660 (Argentina), 2200 (Ala., pt. in Ariz. 4 years previously), 2150 (Texas), 2153 (Ariz.), and 2191 (Ga., pt. in Calif. 10 years previously) were furnished by Dr. Norman F. Conant, Duke Univ. Med. School, Durham, N. Carolina. The fungi were cultured at room temperature at 37°C on the following medium: coconut milk 250 ml; Knop Sol., ½ dil. 500 ml; dist. H_2O 250 ml; trace elements Berthelot sol. 10 drops; dextrose 3%; washed agar 1%; cysteine 10^{-6}; thiamine 10^{-5}; and naphthalene acetic acid 10^{-7}. The chemically defined portion was autoclaved and cooled before adding the coconut milk which had previously been sterilized by filtration. Tubes or flasks of this medium were inoculated on the surface with the mycelium and chlamydospores of *C. immitis* and were incubated at room temperature and at 37°C.

Although growth was evident in 2-3 days, the fungus developed slowly, most of the mycelium being below the surface. The formation of aerial mycelium or of compact leathery mats was prevented by folding such colonies into the medium. Eventually, the fungus produced a soft, grey to tan subsurface growth in which the sporangia developed. Sporangia could be examined microscopically by removing a portion of the growth and mounting it in a drop of lactophenol cotton blue. In such preparations the immature and mature sporangia were found to stain an intense blue in contrast with non-fertile sporangial initials which stained more lightly. In the medium described sporangia were found as early as the 11th day but they usually did not appear until much later—4 to 10 weeks in some cultures. Several tubes inoculated at the same time from the same parent strain gave varying results as regards the age of the culture at which sporangia were produced as well as in the number of sporangia formed. Incubation at 37°C did not appear to hasten sporangial formation. All stages in the development of sporangia and endospores were to be found in mature cultures. Sporangia was formed terminally and intercalarily (Fig. 1, a and b). The majority tend toward a spherical shape but occasionally weirdly shaped sporangia occurred (Fig. 8). Cleavage planes (Fig. 2 and 3) were more clearly evident than is usual in those sporangia found in host tissues and in exudates. The more normal or spherical sporangia ranged in size, when mature, from 5-30 μ in diameter and contained varying numbers of endospores which were usually, but not necessarily, of similar size (Fig. 1c, 4, 5 and 6). Newly released endospores were thin-walled and sometimes vacuolated. Preliminary study indicated that, although these spores may enlarge directly to form sporangia as in host tissues, they frequently germinated to yield hyphae which, in turn, quickly formed more sporangia (Fig. 7, a and b). The thickness of the sporangial wall was found to vary: in those sporangia formed in very young cultures the walls were quite thin while in those sporangia formed in mature cultures the walls were much thicker.

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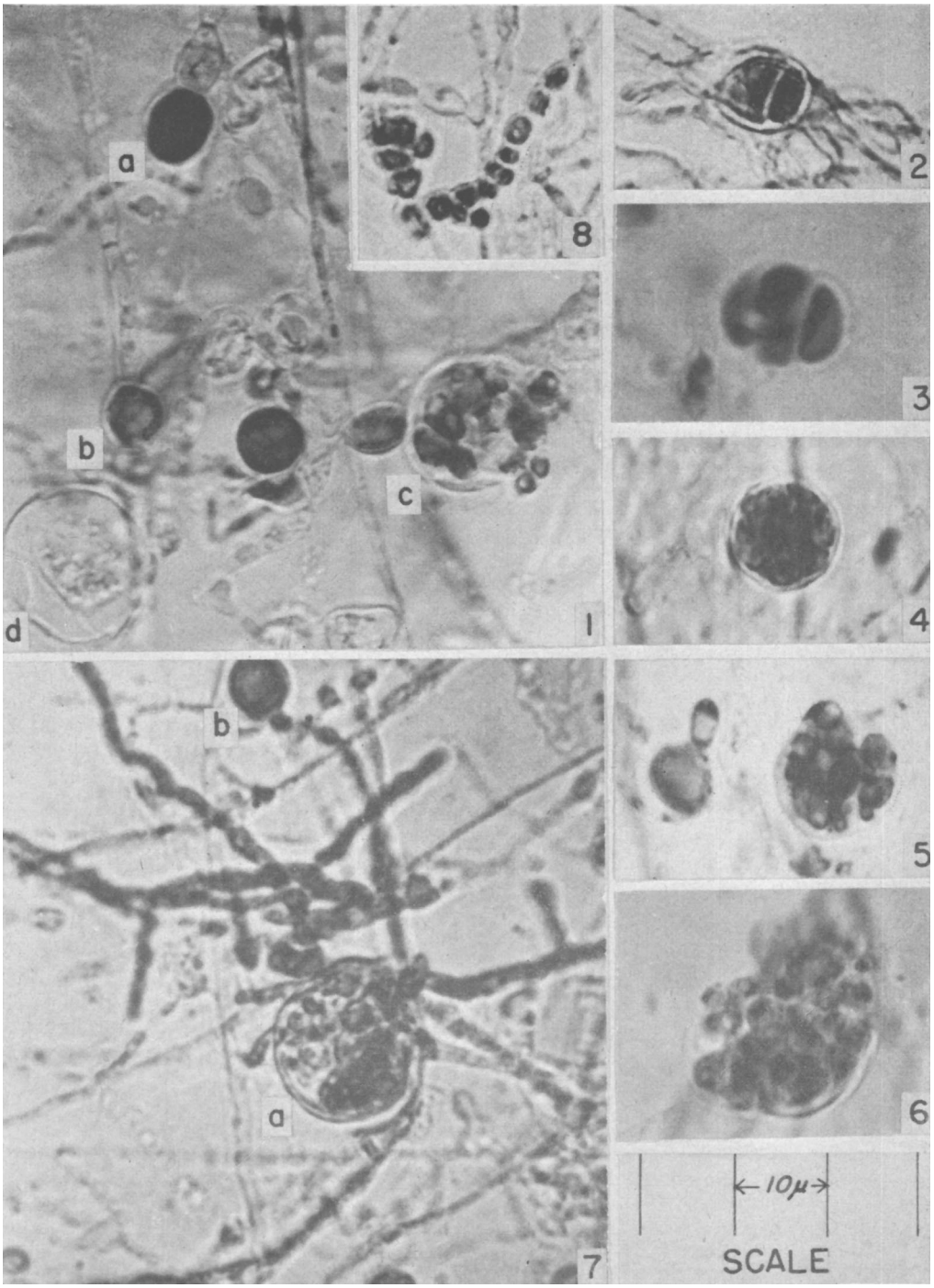

Photomicrographs of material from cultures mounted in lactophenol cotton blue (magnification approx 1300).

FIG. 1. a. Terminal sporangium. b. Intercalary sporangium. c. Mature sporangium releasing endospores. d. Empty sporangium.

FIG. 2 and 3. Early cleavage.

FIG. 4. Mature sporangium containing many small spores.

FIG. 5. Mature sporangium containing many large spores.
FIG. 6. Mature sporangium releasing endospores.
FIG. 7. a. Endospores germinating through sporangial wall. b. Immature sporangium produced by young hypha.
FIG. 8. Hyphal element serving as sporangium.

Further study on certain cytological aspects and on the exact nutritional requirements of the sporangial stage is in progress. Already it has been found that at least one strain will produce sporangia on the basic, chemically defined portion of the medium and on certain fractions thereof, though less satisfactorily than when coconut milk is added.

Summary. The *in vitro* cultivation of the parasitic phase of *C. immitis* is reported and the composition of the medium detailed. Sporangia were formed by all strains studied though in varying numbers and after varying periods of incubation. Mature culture-grown sporangia were indistinguishable from mature tissue sporangia.

Received January 15, 1951. P.S.E.B.M., 1951, v76.

Locus of the Central Emetic Action of Cardiac Glycosides.* (18482)

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It has long been generally accepted that the so-called "central" emetic drugs act by direct excitation of the vomiting center. The concept was especially advanced by Hatcher and Weiss(1) who, in acute experiments, abolished the responsiveness of cats to a variety of emetic agents by making lesions in the dorsal vagal nuclei of the medulla. Also convincing was the demonstration by these workers(1,2) that the direct application of certain "central" emetics to the region of the ala cinerea induced vomiting in dogs and cats.

The premise that drugs act directly on the vomiting center is highly contingent on the certainty of the location of this center. That the dorsal vagal nuclei do not constitute the vomiting center was first indicated by the work of Koppanyi(3). He showed that dogs with chronic lesions in the ala cinerea continued to vomit in response to orally administered irritant emetics in spite of a concomitant refractoriness to the "central" emetic, apomorphine. On the basis of prevailing thought, Koppanyi interpreted his findings as due to incomplete exclusion of the vomiting center. The location of the vomiting center and its physiological role have been adequately delineated only recently by Borison and Wang(4) and Wang and Borison(5). They have demonstrated that the vomiting center, localized in the lateral reticular formation of the medulla, is quite distinct both anatomically and physiologically from the superficial region of the ala cinerea which was shown by Hatcher and Weiss to be sensitive to direct application of certain emetics and which was erroneously designated by them

* This project was supported in part by a grant from the National Institutes of Health, U. S. P. H. S., and in part by funds contributed by Sandoz Chemical Works, Inc.

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