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.

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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.

acid which is formed during the process. A drop of caprylic alcohol was introduced to prevent foaming. Ketene gas was passed through the serum for 35 min at the rate of 100 bubbles per min (diam of inlet tube, 4 mm). At the end of the ketenizing process the pH of the serum was 7.8. Both the ketene treated and the original antisera were diluted with 0.85% physiological salt solution until 1 unit was contained in 0.5 cc. In this test a constant amount of serum and varying amounts of culture were used.

A New York State Laboratory strain, pneumococcus type I "N" (Neufeld) bacterial collection No. 1 was employed. The first blood-broth transfer of a heart-blood culture from a mouse dying within 48 hr after intraperitoneal inoculation of 0.00000001 cc of culture was used in the test. Organisms were cultured in double infusion blood-broth for 16 hr at 37°C. All dilutions were made in broth so that the desired amount of culture was contained in 1.0 cc.

On the assumption that each pneumococcus colony represents the growth from one organism, the number of pneumococci per cubic centimeter was determined by triplicate blood agar pour plates at the time of the test by the use of 1.0 cc each of 10^{-7}, 10^{-8}, and 10^{-9} dilutions of the culture; the colonies were counted after 48 hr incubation.

All dilutions of cultures were made and all mice were injected within 1 hr from the time the dilutions of cultures were prepared. The diluted cultures containing varying amounts of organisms, were thoroughly mixed with diluted serum and 1.0 cc of the mixture immediately injected intraperitoneally into mice weighing from 18-22 g. Each cc of the mixture contained 1 unit of antiserum.

TABLE I.
Influence of Ketene on the Protective Antibody of Antipneumococcus Serum.

Serum	No. of mice used	Unit of serum used (0.5 cc)	cc of culture used	No. of organisms injected	Results
Ketenized 35 min.	9	1	0.5×10^{-3}	4500000	All protected
	9	1	0.2×10^{-3}	1800000	" "
	9	1	0.05×10^{-3}	450000	" "
Original serum	5	1	0.5×10^{-3}	4500000	Four protected. One died in 75 hr
	5	1	0.2×10^{-3}	1800000	Four survived. One died in 85 hr
	5	1	0.05×10^{-3}	450000	All protected
Control	3	0	1.0×10^{-9}	9	All died within 48 hr
	3	0	1.0×10^{-8}	90	" " " 24-30 hr
	3	0	1.0×10^{-7}	900	" " " 16-20 "

Mice surviving for 96 hours or more were considered protected by the serum. The results of this test are given in Table I.

Twenty-seven mice were protected when injected with a mixture of one unit, or 0.005 cc of ketenized serum and pneumococci numbering from 450,000 to 4,500,000, whereas out of 15 mice injected with one unit of original antiserum and a similar number of organisms one died in 75 hours, another in 85 hours. Nine unprotected control mice died within 16 to 48 hours after injection with from 9 to 900 organisms.

The results of the present study indicate that the protective antibody of antipneumococcus horse serum is unchanged when treated with ketene, and strongly suggest that the ketenization of antisera might be very useful in serum therapy.

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Delayed Sedimentation in Antihemocyanin-Systems.

SANFORD B. HOOKER.

From the Evans Memorial, Massachusetts Memorial Hospitals, Boston.

In the course of some turbidimetric observations on precipitative systems, using the Evelyn photoelectric photometer, it became desirable to correlate the measurements with particulation and evenness of sedimentation as determined by direct inspection. Within a certain limited range of rather strong mixtures of *Limulus* hemocyanin and its antiserum it was found that sedimentation was enormously delayed, sometimes requiring several days for completion. The same kind of behavior was noted with the hemocyanin of *Fulgur carica* but not with crude horse serum and its comparatively weak antiserum. If the mixtures were agitated by convective currents, when the tubes were partly immersed in a waterbath, no zone of delayed sedimentation was observed. In weaker mixtures, even though they covered the same range of optimal flocculation, sedimentation proceeded also in the regular way.

One-milliliter volumes of mixtures of a constant amount of antiserum with decreasing quantities of antigen were pipetted into small tubes, 67 x 4.8 mm inside, which were arranged perpendicularly, protected from jar and agitation as far as possible, and kept at room-temperature (*ca* 23°C). In those tubes in which sedimentation was