Survival of *Histoplasma capsulatum* in Experimental Histoplasmosis in Mice.* (22279)

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Previous studies(1,2) have demonstrated that fatal infections following intraperitoneal inoculation of white mice with yeast phase *H. capsulatum* occur within a 30-day period. After this "acute" period very few of the survivors died within the ensuing year. This study was conducted to determine the fate of *H. capsulatum* after inoculation into white mice.

Materials and methods. Three groups of white Swiss male mice were used in these studies. The intraperitoneal route of inoculation was used throughout. At 8 weeks of age one group received 7 million heat-killed cells, while a second group was inoculated with 0.7 million live yeast-phase organisms in 0.5 ml of mucin, as previously described(2). Following a 6-week period, the "immunized" group and survivors of the second group were challenged with 35 million organisms as was a third control group of 14-week-old mice. At various intervals up to 53 weeks after infection, 8 to 14 mice were sacrificed and cultures obtained. From each animal the whole spleen, heart, right kidney and right lung, and a 1 cm portion of liver were removed, aseptically. Each organ was ground in a sterile mortar containing 2 ml of sterile physiologic saline, after which 1 ml of the ground tissue was inoculated on each of 2 Sabouraud's agar plates containing 80 units and 0.5 mg per ml each of penicillin and streptomycin, respectively. The plates were sealed with parafilm, incubated at room temperature, and read at 15 and 30 days. Those cultures showing a solid growth of *H. capsulatum* were arbitrarily designated 4^+, heavy but not solid growth as 3^+, more than 40 colonies as 2^+ and less than 40 as 1^+. The sum of the concentrations divided by the number of plates counted was designated as the ARC (average relative concentration of organisms).

Results. Table I shows the results obtained in mice receiving a single challenge dose of 35 million organisms (Group I). During the first 5 weeks all of 14 mice showed positive cultures from the lungs, liver and spleen, while the heart's blood and kidney yielded 11 and 10 positive cultures, respectively. A relatively higher concentration of organisms was observed in the spleen (2.7), liver (2.4) and kidney (2.0) than in the lung (1.7) and heart's blood (1.5). Of 12 mice sacrificed between 6 to 10 weeks after inoculation, positive cultures were obtained from the spleen and liver in 11 and the kidney in 10, while the heart and lungs yielded only 5 and 2 positive cultures, respectively; the average concentration of organisms was likewise higher in the reticuloendothelial tissues (2.6 to 3.2) as compared to that observed in the heart's blood (1.3) and lung (1.0). A similar relationship was observed in 16 mice between 11 to 15 weeks except for only 5 positive cultures from the kidney as compared to 12 and 14 from the spleen and liver, respectively. After 16 weeks no organisms were recovered from the lungs of 38 mice studied while only 1 showed a positive heart's blood culture. This was in contrast to the kidneys, liver and spleen which yielded 42.8, 21.4 and 35.7% positive cultures, respectively, between 16 to 30 weeks, and 35.7, 21.4 and 14.3%, respectively, between 31 to 45 weeks. During the 46- to 53-week period positive cultures were obtained only from the spleen and kidney of 1 of the 10 mice necropsied.

The results in mice previously "immunized" with 7 million heat-killed cells (Group II) were essentially similar as can be seen in Table I. Again it was noted that after 16 weeks the lungs and heart's blood had been cleared of the organisms. The only suggested difference was the relative decrease of the average concentration of organisms in the

* This investigation was supported by a research grant from the National Institute of Health, Public Health Service.

TABLE I. Survival of *H. capsulatum* in Mice following Primary Challenge, Reinfection, and after "Immunization."

Tissue	Wk post-inoc.	Positive cultures Group I* No.†	%	ARC‡	Group II* No.	%	ARC	Group III* No.	%	ARC
Heart	1– 5	11/14	78.6	1.5	6/8	75.	1.7	13/15	86.7	2.1
	6–10	5/12	41.7	1.3	3/8	37.5	1.3	6/12	50.	1.7
	11–15	2/16	12.5	1.0	1/8	12.5	0	0/ 5	0	0
	16–30	0/14	0	0	0/9	0	"	1/ 7	14.3	1.0
	31–45	1/14	7.1	1.0	0/8	"	"	—	—	—
	46–53	0/10	0	0	—	—	—	—	—	—
Lung	1– 5	14/14	100.	1.7	7/8	87.5	1.9	13/15	86.7	2.0
	6–10	2/12	16.7	1.0	2/8	25.	1.5	6/12	50.	1.7
	11–15	5/16	31.3	"	"	"	1.0	2/ 5	40.	1.0
	16–30	0/14	0	0	0/9	0	0	1/ 7	14.3	"
	31–45	"	"	"	0/8	"	"	—	—	—
	46–53	0/10	"	"	—	—	—	—	—	—
Kidney	1– 5	10/14	71.4	2.0	7/8	87.5	2.0	14/15	93.3	1.6
	6–10	10/12	83.3	3.1	6/8	75.	2.3	9/12	75.	1.9
	11–15	5/16	31.3	2.0	2/8	25.	1.5	3/ 5	60.	2.5
	16–30	6/14	42.8	1.7	1/9	11.1	1.0	3/ 7	42.8	2.0
	31–45	5/14	35.7	1.8	2/8	25.	1.5	—	—	—
	46–53	1/10	10.	1.0	—	—	—	—	—	—
Liver	1– 5	14/14	100.	2.4	8/8	100.	3.0	14/15	93.3	3.4
	6–10	11/12	91.7	2.6	7/8	87.5	1.9	10/12	83.3	3.0
	11–15	14/16	87.5	1.6	4/8	50.	1.3	3/ 5	60.	1.5
	16–30	3/14	21.4	"	2/9	22.2	1.0	3/ 7	42.8	1.3
	31–45	"	"	1.3	1/8	12.5	"	—	—	—
	46–53	0/10	0	0	—	—	—	—	—	—
Spleen	1– 5	14/14	100.	2.7	8/8	100.	3.3	15/15	100.	3.3
	6–10	11/12	91.7	3.2	"	"	2.5	11/12	91.7	2.5
	11–15	12/16	75.	1.8	5/8	62.5	1.8	3/ 5	60.	1.5
	16–30	5/14	35.7	1.6	3/9	33.3	1.7	5/ 7	71.4	2.0
	31–45	2/14	14.3	1.0	1/8	12.5	1.0	—	—	—
	46–53	1/10	10.	1.0	—	—	—	—	—	—

* I = Primary challenge with 35 million organisms. II = Challenge 6 wk after dose of 7 million heat-killed organisms. III = Challenge 6 wk after dose of 0.7 million organisms.
† Numerator = No. positive cultures. Denominator = Total No. cultured.
‡ Avg relative concentration of organisms (see text).

liver (3 to 1.9) and spleen (3.3 to 2.5) between 6 to 10 weeks, as compared to that observed between 1 to 5 weeks; this observation was in contrast to the results noted in Group I where average concentrations in the liver rose slightly from 2.4 to 2.6, and in the spleen from 2.7 to 3.2 (Table I).

Similar results were obtained in the mice which previously had survived sublethal infections with 0.7 million organisms (Group III, Table I). Here again as in Group II there seemed to be the trend downward at 6 to 10 weeks as compared to 1 to 5 weeks in the average concentration of organisms in both the liver and spleen from 3.4 to 3.0 and 3.3 to 2.5, respectively. In this group which had received 2 inoculations of viable organisms, the liver and spleen had 42.8 and 71.4% positive cultures, respectively, between 16 to 30 weeks. During this same time interval the liver had about 22% and the spleen about 35% positive cultures in both Groups I and II.

Discussion. Mortalities in white mice following intraperitoneal inoculation of yeast phase *H. capsulatum* in mucin usually occur within 30 days(1,2). Although deaths from specific infection rarely occur after this period it is evident that organisms are still present in the heart's blood and lungs as long as 11 to 15 weeks after inoculation. The spleen, liver and kidney show positive cultures as late as 45 weeks post-inoculation. Relatively little difference was noted between mice receiving primary challenge (Group I) as compared to those receiving heat-killed organisms

(Group II) or sub-lethal doses (Group III) 6 weeks prior to challenge. The only suggested differences were: 1) an almost 2-fold greater number of positive liver and spleen cultures between 16 to 30 weeks in Group III as compared to the other 2 groups; this could probably be attributed to the residual viable organisms from the inoculation made 6 weeks prior to challenge. 2) A relatively higher concentration of organisms in the liver and spleen between 6 to 10 weeks in Group I as compared to the other 2 groups. When compared to the concentrations of organisms observed between 1 to 5 weeks, there was a definite trend downward in the previously infected or "immunized" mice as compared to the relative rise at 6 to 10 weeks in the primary challenge group.

Summary. Yeast phase *H. capsulatum* when inoculated, intraperitoneally, into white Swiss male mice may be recovered frequently from reticuloendothelial tissues as late as 45 weeks after infection. The lungs and heart's blood usually clear within 16 weeks post-inoculation. Mice surviving previous sub-lethal challenge or those previously given 1 dose of heat-killed organisms show little variation in these respects, when challenged, from those receiving primary challenge.

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Received February 6, 1956. P.S.E.B.M., 1956, v91.

Inhibition of Immunological Enhancement by Endotoxin in Refractory Rabbits. Immunochemical Study.* (22280)

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When Gram negative bacterial endotoxins are repeatedly administered by vein over short periods of time, resistance to the reactions normally produced by these toxic complexes rapidly develops(1). Quantities of endotoxin originally productive of vigorous systemic adjustments no longer produce untoward reactions(2,3). For example, febrile responses normally produced are either obliterated completely(2) or modified. Hemorrhagic and necrotizing phenomena normally produced in tumor tissue and skin prepared for the local Shwartzman reaction do not occur(3,4) after intravenous injection of toxin in refractory rabbits. The generalized Shwartzman reaction is no longer produced by paired spaced intravenous injections of toxin, and the leukopenia producing action of endotoxin is obliterated(5). In addition, refractoriness to the lethal effect of endotoxins occurs(6). Refractoriness to most of these reactions develops very soon after intravenous injections of endotoxin are begun. Resistance to the lethal effect of toxin may be demonstrated as early as 24 hours following the first intravenous injection(7). Refractoriness to each of the other effects of endotoxin occurs within a few days(2-4). This development has been dissociated from the classical immune response primarily by its lack of specificity(8), rapidity of development(7), shortness of duration(2), and lack of correlation with appearance and level of circulating antibody(9,10). Further, agammaglobulinemic patients unable to produce demonstrable circulating antibody develop refractoriness to febrile and intoxicating actions of endotoxins just as readily as do normal persons(11). On

* Aided by grants from the U.S. Public Health Service, American Heart Assn., Minnesota Heart Assn., Helen Hay Whitney Foundation, and Minnesota Division of American Cancer Society.

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