Some Factors Affecting Resistance of Mice to Experimental Histoplasmosis.* (20837)

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Relatively little information is available concerning the factors which are involved in innate and acquired immunity in histoplasmosis. Campbell and Saslaw have reported that white male mice were more susceptible to fatal infection than female mice(1). Salvin described a marked increase in resistance of mice to intracerebral challenge 6 days after immunization with acetone-dried yeast phase *H. capsulatum*(2). Farrell, Cole, Prior, and Saslaw have shown that although dogs inoculated intratracheally with mycelial phase organisms developed no clinical disease, they were resistant to subsequent challenge with mycelial phase plus cortisone which combination produced severe disease in control dogs (3).

It is the purpose of this report to describe the effects of age, sub-lethal infection and immunization on the relative resistance to fatal infection.

Materials and methods. White Swiss male mice were used throughout these studies. Intraperitoneal inoculations of 0.7, 7, 35, 70, 110 or 140 million yeast phase organisms† in 0.5 ml of 5% hog gastric mucin were employed as previously described(1). The mice were observed for a 30-day period, and necropsy performed on those dying during this interval. For immunizing antigens, thrice-washed yeast phase cultures were suspended in saline in concentrations of 35, 7, and 0.7 million organisms per 0.5 ml. These suspensions were then killed, as determined by subculture, by immersion in a 56°C water bath for 2 hours.

Results. Exp. 1 (Relation of age to susceptibility). A total of 235 mice were used in this preliminary experiment. The variation in numbers in each group was determined by the availability of mice in the designated age groups. As can be seen in Table I, a marked decrease in fatalities was observed in the mice of the 14- and 24-week-old groups (40-44%) as compared to the 80 to 88% mortalities in the 6-, 8-, and 12-week-old mice. The sharpest difference occurred between the 12- and 14-week mice. This was apparently an age rather than weight differential since there was only about a gram difference in average weights between these groups (Table I).

TABLE I. Comparative Susceptibility of Male Mice to Fatal Histoplasmosis According to Age.

Age (wk)	Avg wt (g)	Fatalities	
		No.	%
6	17	20/25*	80
8	19	67/84	79.7
12	23	22/25	88
14	24	23/52	44.2
24	27	10/25	40
6†	17	0/25	0

* Numerator = No. of deaths. Denominator = Total No. inoculated with 35 million yeast phase *H. capsulatum* in 0.5 ml mucin.

† Mucin control—Inoculated with 0.5 ml mucin only.

Exp. 2 (Comparison of 8- and 14-week-old mice). To investigate further the results obtained in Exp. 1, 2 groups of mice, 8 and 14 weeks of age, were simultaneously challenged with varying doses of organisms as can be seen in Table II. Again significantly greater re-

TABLE II. Comparative Fatal Histoplasma Infections in 8- and 14-Week-Old Mice.

Dose (million)	8-wk mice deaths		14-wk mice deaths	
	No.	%	No.	%
140	43/43	100	16/20	80
70	46/50	92	11/20	55
35	67/84	79.8	9/20	45
7	14/42	33.3	4/20	20
.7	4/53	7.5	1/20	5
Mucin controls	0/25	0	0/20	0

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

† Strain G-13, a recent isolate from spontaneous histoplasmosis in a dog kindly supplied by Charlotte Campbell of Army Medical Center, Washington, D.C.

TABLE III. Effect of Heat-Killed Yeast Phase Antigen on Susceptibility of Histoplasma Infection.

Antigen dose (million)	Challenge dose (million)* 35 deaths No.	%	110 deaths No.	%
35	9/43	20.9	15/46	32.5
7	28/94	29.7	25/53	52.8
.7	16/51	31.3	26/46	56.5
Saline control†	21/50	42.0	37/50	74.0

* Admin. 6 wk after "immunization."
† .5 ml saline given instead of antigen.

sistance was noted in the older mice receiving the larger inocula of 140, 70, and 35 million organisms.

Exp. 3 (Effect of immunization). Three groups of 8-week-old mice were vaccinated with 0.7, 7, or 35 million heat-killed organisms and challenged 6 weeks later with 35 or 110 million cells. The results, as can be seen in Table III, indicate that significantly increased protection was noted in the mice receiving the 35 million dose antigen. A lesser degree of protection was afforded by the 7 and 0.7 million antigen dose.

TABLE IV. Results of Reinfection of Mice Following Initial Sub-Lethal Infection.

Initial infection Dose (million)	Deaths No.	%	Reinfection Dose (million)	Deaths No.	%
35	94/109	86	35	2/15	13
7	25/ 74	34	35	4/49	8
.7	6/ 73	8	35	14/67	21
M*	0/ 48	0	35	21/48	43.7
.7	4/ 50	8	110	18/46	39.2
M	0/ 50	0	110	37/50	74.0
7	14/ 42	33.3	70 (killed)	0/28	0

* M = Mucin control.

Exp. 4 (Reinfection studies). In an effort to determine the effect of initial sub-lethal infection on the resistance of mice to subsequent reinfection, the following study was conducted. Mice which survived challenges of 0.7, 7, and 35 million organisms at 8 weeks of age were inoculated with 35 or 110 million organisms 6 weeks later. As can be seen in Table IV, a significant decrease in mortality was observed in the mice previously surviving infection. It was noted during the course of this experiment that the deaths in the reinfection group usually occurred within the first 4 days. In contrast, after primary infections, most fatalities were between the 7th to 22nd days. In order to determine whether this was a sensitization or shock phenomenon, 70 million dead organisms in mucin were inoculated into a group of 28 mice which had previously survived infection, but none succumbed to the challenge (Table IV). Similarly none of 42 previously infected mice were affected by 0.5 ml of a 1:100 dilution of histoplasmin, intraperitoneally. The time relationship of deaths in the various categories studied in this project are presented in Table V.

Discussion. The data presented in these studies suggest that white Swiss male mice 8 to 12 weeks of age are more susceptible to experimental histoplasma infections than those 14 weeks or older. This sharp distinction may be related in part to the fact that sexual maturity is not attained by male mice until after 8 to 12 weeks(4). The increased resistance observed after this developmental phase may be compared to observations in human beings. Parsons and Zarafonetis noted that the incidence of human histoplasma infections was highest during the first year of life and after falling off rapidly reached another peak after the 5th decade(5). Their observations also noted that up to, and including the age of 10, the incidence was the same in both sexes, but after that age the ratio was about 7 to 1 in favor of males. Our previous studies with sexually mature mice also showed a greater susceptibility in males (1). This is in contrast to Salvin's report that no differences in susceptibility were noted between male and female mice(2). However, it is of interest to note that he used sexually immature 35-day-old mice. Additional studies to elucidate further the sex and maturity factors are in progress in this laboratory.

That mice surviving previous infections are more resistant to subsequent reinfection with the same organism is also suggested by these studies. It is recognized that the survivors might represent a more naturally resistant group. However, increased protection was also noted in mice receiving the smaller doses which had previously resulted in a low fatality rate. Actually a different pattern of response

TABLE V. Time of Deaths of Mice Following Reinfection with *H. capsulatum*.

1st inoculum	2nd inoculum	No. deaths	% of deaths after days: 1–4	5–8	9–12	13–16	17–20	21–24	24–29
Live cells (.7-35 million)	Live cells (7-110 million)	37/177	17.0	.6	1.7	.6	1.2	0	0
Dead cells (.7-35 million)	*idem*	122/333	1.7	1.3	15.0	12.9	4.9	1.0	0
Normal saline	*idem*	326/541	1.6	11.3	20.3	13.8	6.2	5.2	1.6
Live cells (.7-35 million)	Dead cells (70 million)	1/ 42	0	2.4	0	0	0	0	0
idem	Histoplasmin (.5 cc of 1:100 dilution I.P.)	0/ 42	0	0	0	0	0	0	0
Mucin	Mucin	0/ 25	0	0	0	0	0	0	0

was noted in the reinfection group in that most of the fatalities occurred within 4 days after challenge as compared to the predominance of deaths between 7 to 22 days after primary challenge. Heat-killed antigens or histoplasmin did not induce such reactions in previously infected mice. The mechanism of this phenomenon is being investigated in this laboratory. The introduction of heat-killed antigen resulted in a significantly lowered mortality rate following subsequent challenge 6 weeks later. It is of interest to note that Salvin, using the intracerebral route, found that protection following immunization was greatest 6 days later, animals at that time withstanding 320 $LD_{50's}$ as compared to 10 $LD_{50's}$ at 24 days. This latter period is even less than the 6-week interval which was employed in these studies.

Summary. White Swiss male mice 8 to 12 weeks of age were more susceptible to intraperitoneal inoculations of yeast phase *H. capsulatum* than mice 14 weeks or more in age. Mice surviving previous sub-lethal infections were more resistant to subsequent challenge 6 weeks later. Vaccination with heat-killed yeast phase antigens resulted in a decrease in the number of fatal infections following massive challenge 6 weeks later.

1. Campbell, C. C., and Saslaw, S., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 51.

2. Salvin, S. B., *J. Immunol.*, 1953, v70, 267.

3. Farrell, R. L., Cole, C. R., Prior, J. A., and Saslaw, S., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 51.

4. Snell, G. D., in *Biology of the Laboratory Mouse,* 1941, P. 59, Roscoe B. Jackson Memorial Laboratory, Blakiston, Phila.

5. Parsons, R. J., Zarafonetis, C. J. D., *Arch. Int. Med.*, 1945, v75, 1.

Received January 12, 1954. P.S.E.B.M., 1954, v85.