

ENHANCEMENT OF HISTAMINE AND ANAPHYLACTIC SHOCK IN MICE BY *BRUCELLA ABORTUS*

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AN INCREASE in susceptibility to histamine¹ and to anaphylactic sensitization² has been observed following the inoculation of white mice with a vaccine of *Hemophilus pertussis* phase I organisms. Experimental infection with *H. pertussis*,³ the establishment of sarcoma 180,⁴ increasing age of the animal,⁵ and treatment with a vaccine of *Brucella abortus* organisms⁶ all result in an altered reactivity to histamine also. However, most experimental agents and conditions^{6, 7} which have been described for the mouse have no effect on the inherent resistance of this animal to histamine. On the other hand, although many diverse substances have been found capable of enhancing the anaphylactic sensitivity,⁸ it should be noted that a parallel increase in susceptibility to histamine does not result. In addition to *H. pertussis* and certain fractions obtained therefrom,⁹ only *B. abortus* has been found capable of significantly altering histamine and anaphylactic sensitization.

In contradistinction to the above findings with *B. abortus*, Abernathy and Spink¹⁰ reported that in their hands neither *B. abortus* nor other strains of brucellae had the capability of decreasing the resistance of the white mouse to histamine. Since the results obtained in our previous study⁶ were apparently significant, it was deemed of interest to reinvestigate and augment the experimental criteria of these studies. It should be emphasized at the outset that the capabilities of *B. abortus* for altering these sensitivities, although significant, are far less than those of *H. pertussis*.⁶

MATERIALS AND METHODS

Mice.—Female white mice of the Swiss-Webster strain were purchased from Tumblebrook Farm. We have used this strain of mouse in almost all of our previous studies. At the start of the experiment each mouse weighed about 18 to 20 grams.

B. abortus Vaccine.—We are indebted to Dr. R. V. Johnston of Pittman-Moore Company, Indianapolis, Indiana, for a generous quantity of vaccine. Each vial of *Brucella abortus* vaccine, desiccated, contained approximately 6×10^{10} viable cells of the attenuated strain No. 19. Immediately prior to use the contents of each vial were reconstituted with either 0.85 per cent saline

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containing Merthiolate 1:10,000 or Coca's fluid. The lipopolysaccharide from *B. abortus* used in some of the experiments was provided through the courtesy of Dr. C. W. Christensen of Difco Laboratories, Inc., Detroit, Michigan.

Sensitization.—Each mouse was given an intraperitoneal (i.p.) inoculation of 0.5 ml. of a mixture containing *B. abortus* vaccine (2×10^9 cells) or the lipopolysaccharide (100 or 200 mcg.) and a heterologous protein antigen, either 1 mg. of recrystallized egg albumin (EA) or 0.03 ml. of horse serum (HS).

Histamine Sensitivity.—On the fourth day after immunization, each mouse received an i.p. inoculation of histamine as the phosphate salt, 36 mcg./gram mouse. This amount of histamine corresponds to approximately the L.D.₁₀₀ for mice treated with *H. pertussis*. Although all mice showed some reaction, the actual result is recorded as mortality (number of mice dead/number of mice injected).

Anaphylactic Sensitivity.—On the tenth, fifteenth, or twentieth day following the initial treatment with brucella, an intravenous inoculation of homologous antigen, 1 mg. EA or 0.1 ml. HS, was given each mouse. Reactions other than actual death were disregarded in the final tabulation, with the mortality results expressed as number of animals dead per number of animals challenged.

RESULTS AND DISCUSSION

Our previous conclusions⁶ indicating that pretreatment with *B. abortus* enhances the reactivity of the mouse to the lethal effects of histamine have been confirmed. As can be seen from the data in Table I, a mortality of about 50 per cent was obtained. This value is of statistical significance. We have previously noted⁶ that the degree of sensitivity obtained with *B. abortus* was not as great as that produced by *H. pertussis*, as the 95 per cent mortality obtained for the latter in these experiments will indicate.

TABLE I. EFFECT OF BRUCELLA ABORTUS ON HISTAMINE SENSITIVITY

DAY	CHALLENGE AGENT	SENSITIZING AGENT				
		B. ABORTUS 2 × 10 ⁹ CELLS	HEATED B. ABORTUS 2 × 10 ⁹ CELLS	H. PERTUS- SIS 6 × 10 ⁹ CELLS	LIPOPOLY- SACCHARIDE B. ABORTUS 200 MCG.	UNVACCI- NATED CON- TROL
4	Histamine phos- phate 36 mcg./ Gm. mouse i.p.	42/83*	5/45	18/19	8/34	0/20

*Denotes number of animals dead per number of animals challenged.

In the above experiments inactivation of *B. abortus* was produced with phenol or Merthiolate. The failure that Abernathy and Spink¹⁰ reported may be attributed to their technique, in which heat-killed *B. abortus* organisms were used as the sensitizing medium. Maitland and colleagues¹¹ have reported that the factor responsible for histamine sensitization in *H. pertussis* is inactivated completely when heated at 80 degrees for thirty minutes. Confirmation was extended by Kind¹² who, although he did not find complete inactivation, did observe a reduction in histamine-sensitizing ability following heating. In an attempt to duplicate more closely the technique of Abernathy and Spink,¹⁰ a

subsequent study was carried out using heat-killed organisms obtained by autoclaving for thirty minutes at 15 lbs. pressure a saline-reconstituted suspension of *B. abortus*. As can be seen, the mortality obtained by prior sensitization with these heat-killed cells was only about 10 per cent, thus confirming the effects of heating on the sensitizing properties.

Since relatively few extraneous agents have the ability to decrease the tolerance of the mouse to histamine, a certain degree of specificity must be recognized. It has been noted that *H. influenzae*,⁶ *H. pertussis* phase IV,⁷ and certain other unrelated vaccines⁶ do not possess the histamine-sensitizing property. The failure to produce an increased sensitivity with *B. melitensis*, *B. suis*, or active infection with *B. melitensis* as reported by Abernathy and Spink¹⁰ should, therefore, not have been entirely unexpected. With reference to the strain difference that exists between mice with respect to ability to sensitize to histamine, it should be indicated that this has been amply demonstrated.^{13, 14}

The observation that prior treatment of the mouse with a lipopolysaccharide obtained from *B. abortus* produces a decreased tolerance to histamine (Table I) also is of considerable interest. It should be recalled that an increased sensitivity to histamine could not be demonstrated by us⁸ using the protein nucleate of *B. abortus*, or by Abernathy and Spink,¹⁰ using brucella endotoxin.

TABLE II. EFFECT OF BRUCELLA ABORTUS ON ANAPHYLACTIC SENSITIZATION TO HORSE SERUM AND EGG ALBUMIN

DAY	CHALLENGE AGENT	SENSITIZING AGENT							
		B. ABORTUS 2 × 10 ⁹ CELLS		HEATED B. ABORTUS 2 × 10 ⁹ CELLS	H. PERTUS- SIS 6 × 10 ⁹ CELLS	LIPOPOLY- SACCHARIDE B. ABORTUS (EA)		UNVACCI- NATED CON- TROL	
		HS	EA	HS	HS	100	200	HS	EA
						MCG.	MCG.		
10	HS 0.1 ml. i.v. EA 1 mg. i.v.	2/62*		1/38	14/14			0/15	
			0/24			14/15	20/33		0/20.
15	HS 0.1 ml. i.v. EA 1 mg. i.v.	11/55		4/47				3/66	
			5/10						
20	HS 0.1 ml. i.v. EA 1 mg. i.v.	2/21							
			0/20						

*Denotes number of animals dead per number of animals challenged.

The capability of *B. abortus* to enhance anaphylactic sensitization in the white mouse was re-evaluated and confirmed our previous observations.⁶ The results are shown in Table II. Sensitization seems to reach a maximum at about the fifteenth day and subsequently decreases rapidly. Using *H. pertussis* as the sensitizing organism, our experience has shown that the height of anaphylactic sensitivity is reached at about the tenth day and persists at a

maximum for an additional ten days. These differences, together with the lower incidence of mortality obtained with *B. abortus*, further emphasize that the latter is not as potent a conditioner to sensitization as is *H. pertussis*.

It is of interest that the lipopolysaccharide of *B. abortus* enhances anaphylactic sensitization. At the 200 meg. level a mortality of about 60 per cent was obtained by the tenth day, while at the 100 meg. level a 93 per cent mortality was attained. The difference in mortality at the two levels may be partly the effect of the toxicity of the lipopolysaccharide; at the higher dosage levels the mice became toxic, with many deaths resulting following inoculation with the polysaccharide. The decreased incidence of mortality following challenge may perhaps be a reflection of the stress phenomenon. The isolation of a nonparticulate, soluble fraction which enhances sensitivity offers a valuable tool for furtherance of the investigation into the mechanisms involved.

SUMMARY

The ability of *Brucella abortus* organisms to alter the sensitivity of the white mouse to the lethal effects of histamine has been confirmed. It has been shown that heating has the effect of decreasing this property. Anaphylactic sensitization is also diminished by heating. The lipopolysaccharide obtained from *B. abortus* was also observed to have the capability of sensitizing the white mouse to the lethal effects of histamine and protein antigens.

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