

Anaphylaxis in *Peromyscus leucopus* mice

Saul Malkiel, Ph.D., M.D., and Betty J. Hargis, Ph.D., Boston, Mass.

*Five strains of laboratory mice (*Mus musculus*) were readily sensitized by the pertussis technique; an anaphylactic mortality of 60 to 100 per cent resulted following challenge. By contrast, only a 30 per cent mortality was obtained for the common white-footed deer mouse (*Peromyscus leucopus*). Although the total adrenal gland weight for the latter species is about 2 to 3 times that of the former, the plasma corticosterones and total catecholamines content of the gland were essentially the same.*

Various strains of white mice differ markedly with respect to their capacity to become sensitized to the lethal effects of histamine following treatment with *Bordetella pertussis* vaccine.¹

No such difference has hitherto been reported for the development of active anaphylaxis by pertussis stimulation. The mechanism for the enhancement of anaphylactic susceptibility of the laboratory mouse (*Mus musculus*) to antigenic challenge is yet to be understood.² Some of the evidence which indicates that the adrenal glands participate in the reaction includes: Anaphylactic mortality is lowered in mice pretreated with cortisone,³ adrenalectomy renders the mouse exquisitely sensitive to challenge,⁴ cortisone diminishes the susceptibility of the adrenalectomized mouse to anaphylactic sensitization,⁴ and it protects the adrenalectomized mouse from histamine death.⁵

The common white-footed wild deer mouse, *Peromyscus leucopus*, is an interesting animal in which to study anaphylactic sensitization for it possesses exceptionally large adrenal glands.⁶ The present report discusses the anaphylactic reaction of this genus and, also, several strains of the usual laboratory mouse.

MATERIALS AND METHODS

Mice

Groups of the following were used: (1) *P. leucopus*,* (2) LAF₁, (3) A/JAX (both these species were purchased from the Roscoe B. Jackson Memorial

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Laboratory, Bar Harbor, Maine), (4) CFW, (5) CF1 (both these species were purchased from Carworth, Inc., New City, New York), and (6) C3H (bred for several generations in this laboratory). The groups consisted of from 5 to 30 animals, each weighing about 18 to 20 grams at the start of the experiment. They were maintained on Purina Laboratory Chow and allowed drinking water ad lib.

Sensitization

A single intraperitoneal (i.p.) injection of a mixture of 6×10^9 *B. pertussis* phase I organisms and either 0.03 ml. of horse serum (HS) or 1 mg. of crystalline bovine serum albumin (BSA) contained in 0.5 ml. of saline solution was given each mouse as the immunizing dose. As controls, mice received an i.p. injection of antigen only, without pertussis cells.

Challenge

The mice of each group received 0.1 ml. of horse serum or 1 mg. of BSA in 0.1 ml. of saline solution intravenously (i.v.) as the challenge dose 10 days after sensitization. The BSA-sensitized animals received a secondary challenge 7 days following the primary challenge. The mortality for each group was recorded 24 hours postchallenge.

Histology

The adrenal glands were removed from killed normal *P. leucopus* and CFW mice, weighed, fixed in 10 per cent formalin, sectioned, stained with hematoxylin-eosin, and examined microscopically.

Catecholamines

The total catecholamine content of the adrenal glands obtained from normal mice was quantitated by a modification⁷ of the method of Anton and Sayre.⁸

Corticosterone

Peripheral blood was obtained from normal animals by puncture of the ophthalmic venous plexus. The level of corticosterone was determined on pools of plasma from *P. leucopus* and CFW mice by the method of Moneloa and colleagues.⁹

RESULTS

Anaphylaxis

The results, which are delineated in Table I, indicate that challenge with HS evoked a mortality of about 95 per cent in the pertussis-sensitized CFW but only 30 per cent in the *P. leucopus* mouse. Control mice receiving only HS as the sensitizing antigen gave a 0 and 10 per cent mortality response, respectively. The mortality range for the other strains ranged from 60 to 100 per cent for the pertussis-treated groups. It should be noted that the A/JAX control mice showed an 80 per cent mortality; the other control strains failed to show any significant mortality.

The primary challenge of the pertussis-treated *P. leucopus* mouse with BSA

Table I. *Anaphylactic sensitization in various strains of mice*

<i>Sensitizing antigen</i>	<i>CFW</i>	<i>CF1</i>	<i>A/JAX</i>	<i>C3H</i>	<i>LAF₁</i>	<i>P. leucopus</i>
<i>Challenge antigen—HS*</i>						
HS†	$\frac{9}{10}$ ‡	$\frac{9}{10}$	$\frac{8}{10}$	$\frac{9}{10}$	$\frac{1}{5}$	$\frac{1}{10}$
Pertussis-HS	$\frac{17}{18}$	$\frac{7}{11}$	$\frac{19}{19}$	$\frac{8}{8}$	$\frac{3}{5}$	$\frac{3}{10}$
<i>Challenge antigen—BSA§</i>						
	<i>Primary Secondary-ary¶</i>				<i>Primary Secondary-ary</i>	
BSA	$\frac{9}{20}$	$\frac{4}{10}$			$\frac{9}{10}$	$\frac{9}{10}$
Pertussis-BSA	$\frac{9}{20}$	$\frac{6}{8}$			$\frac{5}{30}$	$\frac{18}{24}$
					$\frac{2}{8}$	$\frac{1}{6}$

*Challenge antigen, 0.1 ml. intravenously 10 days after sensitization.

†HS = horse serum, 0.03 ml. intraperitoneally with or without *B. pertussis*, 6×10^9 organisms.

‡Lethality: number of animals dead per number challenged at 24 hours.

§Challenge antigen, 1 mg. intravenously 10 days after sensitization.

||BSA = bovine serum albumin, 1 mg. intraperitoneally with or without *B. pertussis*.

¶Secondary challenge 7 days after primary.

Table II. *Weights of paired adrenal glands and total adrenal catecholamines of various mice*

	<i>CFW</i>	<i>CF1</i>	<i>A/JAX</i>	<i>C3H</i>	<i>LAF₁</i>	<i>P. leucopus</i>
Average adrenal weight (mg./pair)	7.8	4.7	2.6	3.2	3.7	14.0
Average total catecholamines (μ g)/adrenal pair	7.4	6.5	3.8	4.0	6.0	4.5
Average total catecholamines (mg.)/gram of tissue	1.5	1.5	1.5	1.3	1.7	0.4
Average/species			1.5*			0.4*

*This difference is significant: $P = 0.001$.

resulted in a 25 per cent mortality rate, with CFW 25 per cent and LAF₁ 17 per cent. Secondary challenge of the surviving mice produced 17 per cent, 75 per cent, and 75 per cent mortalities, respectively.

Histology

The weights of paired adrenal glands can be found in Table II. Those from *P. leucopus* ranged from 6.0 to 31.0 milligrams, the average being 14.0 (55.0 to 125 milligrams per 100 grams), and from CFW paired adrenal weights varied from 2.7 to 13.8 milligrams, the average being 7.8 (20.0 to 53.8 milligrams per 100 grams). The average weights for the other species of laboratory mouse ranged from 2.6 to 4.7 milligrams.

Histologically there seem to be striking features which differentiate the adrenal glands of the *P. leucopus* from the CFW mouse. The regions of the zona

glomerulosa are similar in that they are relatively thin and poorly defined. Some cells of the zona fasciculata of the adrenals of *P. leucopus* mice show the presence of large nuclei which appear to be three times the volume of the numerically predominant nuclei. The zona reticularis is relatively thicker. In this zone there is an even more striking difference in nuclear volume, ranging from relatively small nuclei through medium-sized to giant forms. Between the zona reticularis and the medulla there is a zone of very large vacuoles resembling huge fat cells. The cells of the medulla are essentially identical in both genera.

Catecholamines

The results of these determinations, which are seen in Table II, show that the total catecholamine content per pair of adrenal glands ranged from an average of 3.8 μg for the A/JAX to 7.4 μg for the CFW mice. The adrenal glands of the *P. leucopus* mice averaged 4.5 μg total catecholamines. These figures correspond to a value of 0.4 mg. total catecholamines per milligram of adrenal tissue for *P. leucopus* and from 1.3 to 1.7 mg. for the other mice studied.

The plasma corticosterone level for the *P. leucopus* mice averaged 26 μg per cent while that for CFW mice averaged 24 μg per cent.

DISCUSSION

The data presented show that the different strains of the laboratory mouse, *Mus musculus*, studied by the above experimental procedures were all capable of becoming anaphylactically sensitized to a soluble foreign protein by the use of the pertussis technique. The mortality following challenge with the homologous antigen ranged from 60 to 100 per cent. No strain difference was observed with the possible exception of the A/JAX mouse. An 80 per cent mortality rate followed sensitization with a single dose of antigen alone; this value reached 100 per cent by incorporating *B. pertussis* with the immunizing antigen. The other strains failed to display any significant anaphylactic mortality from a single dose of antigen alone. Tokuda and colleagues¹⁰ reported a strain difference following challenge of the pertussis-vaccinated mouse with soluble antigen-antibody complexes. However, this procedure is an example of a passive rather than an active anaphylaxis and quite likely involves a separate mechanism. In addition, these authors¹⁰ have confirmed our results¹¹ indicating that mice sensitized with a pertussis-antigen mixture are much more reactive to anaphylactic challenge than those treated with Freund's adjuvant. Rothberg and Talmage¹² concluded that the severity of anaphylaxis appeared related to the amount of circulating antibody as determined in three strains of mice using Freund's adjuvant as the sensitizing mixture. By this technique the CF1 mouse was found to produce the most antibody and was most reactive to anaphylactic challenge; this is the strain which is markedly resistant to sensitization to histamine even after pertussis¹ or adrenalectomy.¹³

Farr and colleagues¹⁴ have indicated that the capability to produce antibody is under genetic influence. Although anaphylactic data do not emphasize quantitative antibody differences, this concept is supported by the observations on the behavior of the A/JAX mouse to a single dose of antigen, as discussed above, and

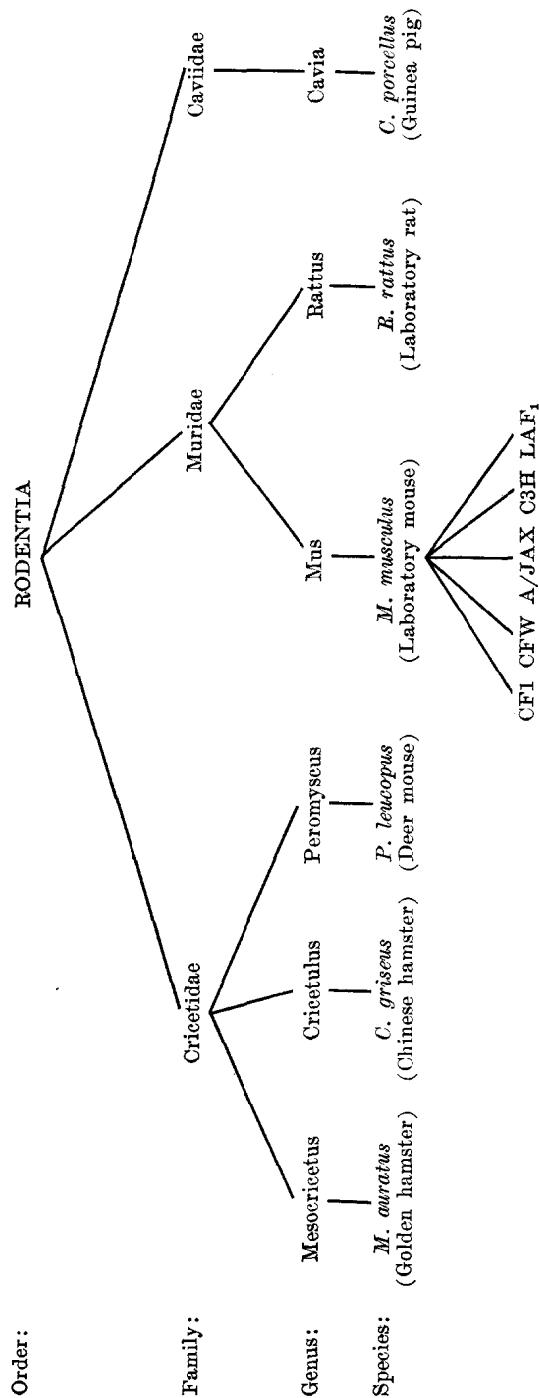


Diagram 1
Family relationship of the commonly used experimental rodents.

the enhanced sensitization by *B. pertussis* of the various strains of *Mus* as compared to *P. leucopus*. An even greater disparity exists among family members of the order Rodentia: anaphylactic sensitization is most facile in the guinea pig, can be accomplished in the laboratory mouse by repeated injections of an antigen, easily results in the mouse and rat by the use of *B. pertussis* with a single dose of antigen, is produced to a lesser degree in *P. leucopus* with pertussis, and does not occur at all in the Chinese and golden hamsters.¹⁵ The relationship of these families is depicted in Diagram 1.

The induction of histamine sensitivity, adjuvant action, enhancement of active anaphylaxis, and other physiological alterations produced by *B. pertussis* have been ascribed¹⁶ to the histamine-sensitizing fraction (HSF), which has been identified as either being identical to or inseparable from the immunogen.¹⁷ However, there is an accumulation of published reports which emphasize the differences between anaphylaxis and histamine sensitivity and tend to suggest that the two phenomena are not related.

The following conclusions should be considered: It has been shown that pertussis treatment does not engender an increase in sensitivity to histamine in all strains of mice.¹ Specifically, no significant enhancement could be demonstrated in the CF1 strain following either pertussis inoculation¹ or adrenalectomy.¹³ Pertussis pretreatment increased the sensitivity of the Taconic strain of the Swiss-Webster mouse whereas surgical adrenalectomy had no significant effect.¹⁸ The Rocky Mountain strain has been found to be resistant to the effects of HSF and adrenalectomy at 3 to 5 weeks of age but becomes susceptible by 8 weeks.¹⁹ All strains of mice develop an anaphylactic sensitivity following inoculation with a *B. pertussis*-antigen mixture. Indeed, the lack of parallelism between anaphylaxis and histamine sensitivity has led to the concept that the mediators may not be involved in the anaphylactic phenomenon to any significant degree.²⁰

From a consideration of the above, it becomes difficult to reconcile the physiological alterations to a single molecule which would be inherent to a unitarian hypothesis.¹⁶ This would assume the presence of specific determinant groupings on HSF directing its many functions. It seems no less tenable to ascribe the antibody-stimulating and anaphylactic-enhancing capacity, at least, to some other fraction of *B. pertussis*. For this aspect the lipopolysaccharidic moiety (LPS) needs be considered, since previous studies have shown that this fraction is antibody-stimulating and anaphylaxis-enhancing.²¹ Further, there are no definitive studies which show that purified HSF will enhance anaphylactic sensitization.

The increased histamine and serotonin susceptibility which results following adrenal demedullation²¹ or treatment with β -adrenergic blocking agents^{23, 24} suggest that the catecholamines are involved. Again, there is some strain disparity, for both *B. pertussis* and the β -blockers increase sensitivity to histamine and serotonin in the CFW strain, but only the β -blockers are effective in the CF1 mouse.²⁵ Also, epinephrine affords protection against the histamine-sensitizing effect of pertussis vaccination.²² Our studies show that there is no essential difference in the total adrenal catecholamines for the strains studied. The difficulty with which *P. leucopus* sensitizes to anaphylaxis and the resistance of this species of mouse to histamine following adrenalectomy¹³ may indicate

that the catecholamines are not involved in this phenomenon. Figures for the corticomedullary relationship of these experimental mice would be of interest in this regard since the adrenal glands of the *P. leucopus* mice are so large; however, these are not available.

The plasma corticosterone levels of both *P. leucopus* and *M. musculus* (CFW strain) were identical despite the marked cortical hyperplasia in the former. The meaning and functional significance of the remarkable anisocytosis in the former species are unknown.²⁶ Perhaps of some relationship is the observation that ACTH increases adrenal weight by causing a cortical hyperplasia and cellular hypertrophy, particularly of the inner portion of cortex.²⁷ The finding of similar levels of plasma corticosterone is consistent with the current concept that the cortical secretions have no significant role in the anaphylactic sensitization.

The experimental results obtained here can be interpreted as indicating that there is a fundamental difference between the phenomena of anaphylactic and histamine sensitization. The mechanism for the former and the death that ensues on challenge are the result of a complex interrelationship involving mediators and enzymes, a dependency upon genetic factors influencing antibody production, shock organ sensitization, etc. It may well be that both HSF and LPS are involved in the anaphylactic process.

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