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Protection Against Histamine Shock by Catecholamines in *Bordetella pertussis*-Treated, Adrenalectomized, or Adrenergic Blocked Mice. (31153)

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The intraperitoneal (i.p.) LD_{50} of histamine for normal mice is between 750-1000 mg/kg body weight. This remarkable resistance to histamine can be decreased in some strains by treatment with the histamine sensitizing factor (HSF) from *Bordetella pertussis,* by removal of the adrenal glands(1,2), or by administration of β-adrenergic blocking agents, such as dichloroisoproterenol (DCI) and nethalide(3). The increased sensitivity is not limited to histamine, since pertussis vaccine and adrenalectomy also increase the susceptibility of mice to serotonin, anaphylactic shock, cold shock and various other forms of stress(2). In addition to their potentiating effects on histamine sensitivity, β-adrenergic blocking agents also increase the susceptibility of mice to serotonin(3).

The exact mechanism by which this hypersensitization phenomenon is brought about is still not completely known. The characteristics of hypersensitization after adrenalectomy or treatment with HSF are quite similar, suggesting that adrenal hormones might be involved in the hypersensitization produced by HSF. Adrenalectomized mice can be protected from histamine shock with cortisone and epinephrine(4,5) and adrenalectomized rats can be protected by epinephrine alone(6). HSF-treated mice have also been protected from histamine shock with large doses of cortisone(7,8) but we have found no report of protection with catecholamines; to the contrary, failure to protect *B. pertussis*-treated mice with epinephrine has been reported(9).

During anaphylaxis and histamine shock in the mouse, there is a profound loss of blood volume as shown by a marked rise in hematocrit values(4,5,9-12). Hypotension is known to be a potent stimulus for the release of endogenous catecholamines in the dog(13). The salutary effects of these hormones are presumably due to their vasopressive actions which improve venous return to the heart and help maintain circulation to vital organs, *e.g.*, heart and brain. The purpose of this investigation was to determine whether catecholamines could protect histamine hypersensitive mice against histamine shock.

Materials and methods. Mice. Equal numbers of 7-8-week-old male and female CFW mice raised in our laboratory were used. During the experimental period, these mice were housed in groups of 5 in glass jars, 7 inches deep by 7 inches in diameter, containing beet pulp bedding and Purina Laboratory Chow. Sexes were kept separated.

Histamine sensitizing factor. HSF was given as a dialyzed and lyophilized alkaline saline extract (SE) of acetone-dried *B. pertussis* cells(14).

Drugs. L-epinephrine, dl-epinephrine, dl-norepinephrine-HCl (dl-arterenol-HCl), l-ephedrine alkaloid and dl-ephedrine HCl were obtained from Sigma Chemical Co. Histamine was given as histamine diphosphate (Nutritional Biochemicals Corp.). Dichloro-

isoproterenol (Eli Lilly) and nethalide (Alderlin, Imperial Chemical Industries, Ltd., Great Britain) were used for β-adrenergic blocking agents. Angiotensin amide (Hypertensin, Ciba Pharmaceutical Co.) and metaraminol (Aramine, Merck, Sharp & Dohme) were also used as vasoactive agents. All drugs were made up to the desired concentration in isotonic saline. However, with l-epinephrine and dl-epinephrine, it was found easier to make an initial dilution in isotonic saline adjusted to pH 2 with HCl, before diluting them to the final concentration. All dosages for drugs are expressed as the base.

Sensitization and challenge procedures. Bilateral adrenalectomies were performed as described previously(15). Adrenalectomized mice were supplemented on 1% NaCl drinking water and allowed at least 24 hours to recover from the operation. Adrenal demedullation was performed as described by Ingle and Griffith(16). A few of the demedullated mice were challenged 2 days later, but the majority were held for 33 days to insure adequate cortical repair. These mice were also given 1% NaCl in their drinking water for the first week. HSF-treated mice were given intravenously (i.v.) the desired quantity of SE in 0.2 ml isotonic saline the day before histamine challenge. β-adrenergic blocking agents were also given i.v. in 0.2 ml saline 15-20 minutes prior to challenge. Histamine challenges were given i.p. in 0.2 ml isotonic saline, unless otherwise indicated.

Protective treatment with catecholamines. Catecholamines were given i.v. in 0.1 ml of isotonic saline within 30 to 60 seconds after histamine challenge. Significance of differences between results obtained with unprotected controls and experimental animals was statistically evaluated from probability tables given by Mainland(17).

Experimental. Initially, experiments were conducted to determine the relative importance of adrenal cortical tissue and medullary tissue on histamine sensitization. It was found that totally adrenalectomized or demedullated mice were equally sensitive to histamine (Fig. 1). In most cases when "demedullated" mice survived the histamine challenge, histological examination revealed significant amounts of

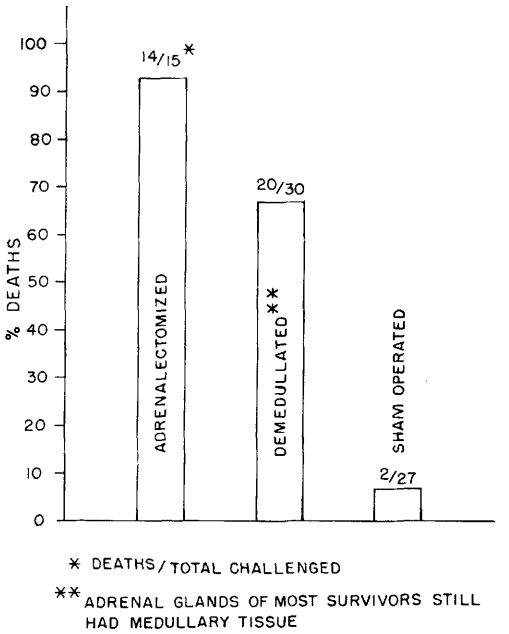


FIG. 1. Sensitivity of adrenalectomized and adrenaldemedullated mice to 0.5 mg histamine.

chromaffin tissue still remaining in the adrenal medullary area(Fig. 2). Cortical tissue in all demedullated adrenals appeared healthy on microscopic examination (Fig. 2). Catecholamines were effective in protecting both demedullated and adrenalectomized mice from histamine challenge (Fig. 3 and 4). Dl-norepinephrine gave some protection against histamine toxicity but the best protection was obtained with 1.25 to 2.5 μg of l-epinephrine given approximately 1 minute after challenge. These experiments suggested that adrenal medullary hormones play an important role in the resistance of normal mice to histamine.

Protection by catecholamines was more difficult to achieve with HSF-treated mice than with adrenalectomized or demedullated mice, perhaps because there appeared to be a competitive relationship between HSF and l-epinephrine. As shown in Table I, 7.5 μg l-epinephrine protected nearly all mice receiving 2.5 or 5.0 μg SE, while those receiving 10.0 or 20.0 μg SE were protected poorly. As dosage of SE was increased, the protection by l-epinephrine was diminished. In all cases, larger quantities of l-epinephrine were required for protection of HSF-treated than for

adrenalectomized or adrenal-demedullated mice.

In the preceding experiments, protection by catecholamines was obtained when histamine challenge was given i.p. and l-epinephrine i.v. 30 seconds later. With this sequence of injections, it was possible that protection could have been due merely to a slower rate of absorption of histamine from the peritoneal cavity. To rule out this possibility, an experiment was performed in which a histamine challenge of 0.2 to 0.4 mg in 0.1 ml was given i.v. 30 seconds before the l-epinephrine treatment. The protection afforded by l-epinephrine in this experiment was even more dramatic than when the challenge was given i.p. (Fig. 5).

Since other workers(9) have failed to protect *B. pertussis*-treated mice from histamine toxicity with 20 or 50 μg of epinephrine-HCl given 30 minutes before challenge, it was important to determine the role of time of treatment with l-epinephrine on its protective activity. Table II demonstrates that 7.5 μg of l-epinephrine given i.v. 10 minutes before or 5 minutes after challenge is not as effective as when it is given 30 seconds after challenge.

The results obtained with l-epinephrine led

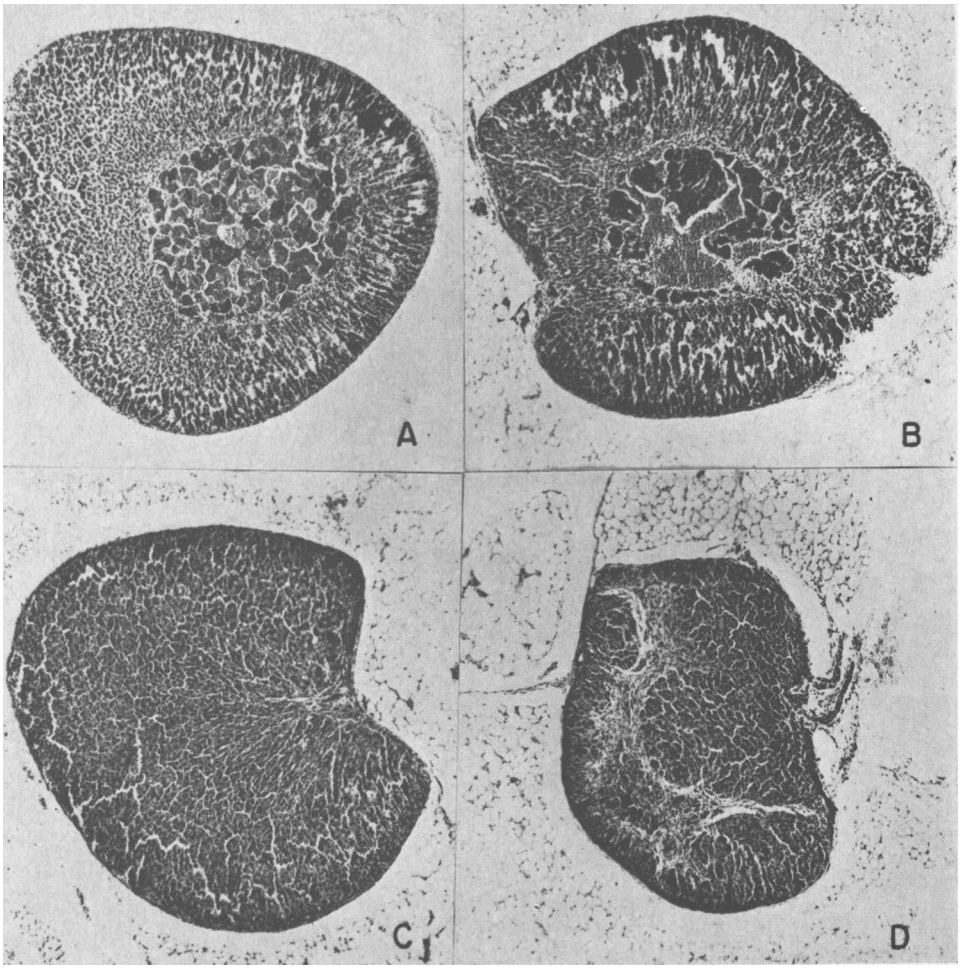
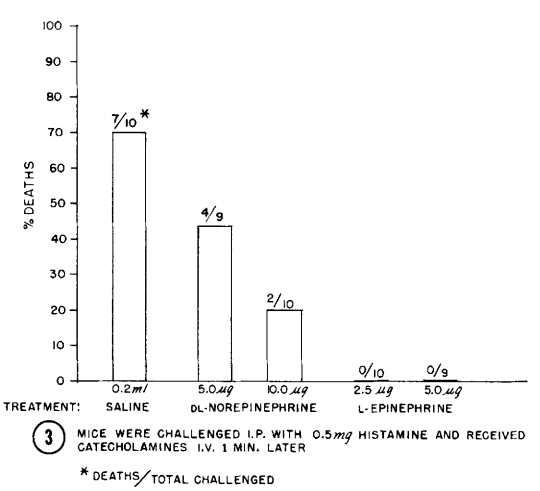


FIG. 2. Cross sections of adrenal glands from sham operated and demedullated mice. A. Adrenal gland from a sham operated mouse which survived histamine challenge. B. Demedullated adrenal gland from mouse which survived histamine challenge (note medullary tissue). C. and D. Demedullated adrenal glands from mice which died from histamine challenge (note absence of medullary tissue).



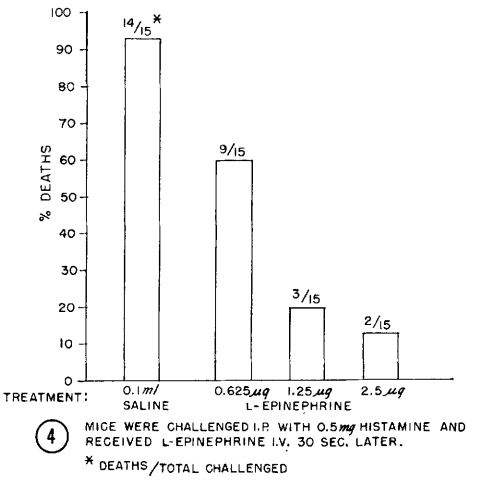


FIG. 3. Protection of adrenal-demedullated mice by catecholamines.
FIG. 4. Protection of adrenalectomized mice by l-epinephrine.

us to test other vasopressive agents for their protective ability, not only on mice made sensitive to histamine by adrenalectomy and HSF-treatment but also by β-adrenergic blocking agents (DCI or nethalide). The results of these studies are summarized in Table III. In all cases (HSF-treated, adrenalectomized or β-adrenergically blocked mice) epinephrine gave significant protection. Dl-norepinephrine was not as effective in HSF-treated mice as it was in adrenalectomized or β-adrenergically blocked mice. The vasopressors, ephedrine, metaraminol and angiotensin were ineffective or gave protection of questionable significance.

TABLE I. Dose Effect of SE Given i.v. on Protecting Ability of l-Epinephrine.

SE, μg/mouse	l-epinephrine treatment in μg/mouse*					
	0†	2.5	5.0	7.5	10.0	12.5
2.5	11/20‡	5/20	5/20	1/20		
5.0	13/20	8/20	6/20	4/20		
10.0	16/20		10/20	11/20	11/20	
20.0	20/20			19/20	15/20	16/20

* All mice received i.v. the dose indicated in a volume of 0.1 ml, and were challenged i.p. with 0.5 mg histamine.
† These mice received i.v. 0.1 ml of isotonic saline.
‡ Deaths/total challenged.

Discussion. The vascular system is probably the shock organ in mice during anaphylaxis or histamine shock(12). During anaphylaxis or after histamine administration, these animals exhibit a marked loss of blood volume and can be protected from death by injections of isotonic saline or 6% dextran solution. The degree of hemoconcentration in normal mice after histamine challenge is con-

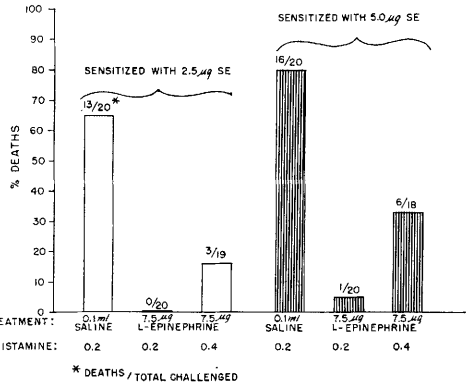


FIG. 5. Protection of HSF-treated mice against i.v. histamine challenge by l-epinephrine given 30 sec after challenge.

TABLE II. Effect of Timing of l-Epinephrine Treatment on Protection Against Histamine Death.

Time of treatment	Treatment	
	0.1 ml saline, i.v.	7.5 μg l-epinephrine, i.v.
10 min before challenge	13/20*	11/20
30 sec after challenge	13/20	5/20
5 min after challenge	12/20	12/20

* Deaths/total challenged.

All mice received i.v. 5.0 μg SE in 0.2 ml saline the day before i.p. challenge with 0.5 mg histamine in 0.2 ml saline.

TABLE III. Effects of Vasopressor Agents Against Histamine Death in Mice Made Hypersensitive to Histamine.

		Hypersensitivity to histamine induced by:							
						β-adrenergic blocking agent‡			
Vasopressor	μg/mouse	Saline extract*		Adrenalectomy†		500 μg DCI		450 μg nethalide	
		D/T§	P‖	D/T	P	D/T	P	D/T	P
None¶		16/20		20/20		15/20		16/20	
l-epinephrine	5.0	8/20	.0112						
	7.5	7/20	.0048						
dl-epinephrine	2.5			9/20	.0001	9/20		5/20	.0006
	5.0			4/20	<.0001	2/20	<.0001	4/20	.0002
	10.0	7/20	.0048						
	15.0	2/20	<.0001						
dl-norepinephrine	10.0	10/20		14/20	.0101	9/20		7/20	.0048
	15.0	9/20	.0242	12/20	.0016	3/20	.0002	2/20	<.0001
l-ephedrine	125.0	17/20							
	250.0	18/20							
dl-ephedrine	250.0	17/20		14/19	.0202	16/20		17/20	
	500.0	20/20		17/18		19/20		14/20	
metaraminol	5.0	16/20		18/18		16/20		19/20	
	7.5	17/20		18/18		14/20		18/20	
angiotensin	5.0			18/18		14/20		14/20	
	7.5	8/15		18/18		15/20		17/20	

* 5 μg of SE was given i.v. on day before challenge.
† Bilateral adrenalectomies were performed on day before challenge.
‡ β-adrenergic blocking agent was given 15-20 min before challenge.
§ Deaths/total challenged.
‖ Treated *vs* untreated controls. According to Mainland the levels of significance are: <.025 = significant; <.005 = highly significant. Except as noted, all other probability values were >.025.
¶ Control mice received 0.1 ml isotonic saline.

siderable, but it is slightly greater in HSF-treated mice.

The ability of normal mice to recover from histamine shock might be due to either the ability of their vascular system to compensate for the blood volume lost or to the fact that they do not lose as much blood volume as sensitized mice.

The data presented here strongly suggest that the enhanced histamine sensitivity seen in adrenalectomized, HSF-treated or β-adrenergically blocked mice is due to impaired catecholamine (especially l-epinephrine) function. The failure of other workers to show protection by l-epinephrine(9) may have been due to the timing between challenge and treatment, since the vasoactive effects of epinephrine last only for a few minutes(18).

HSF does not appear to prevent the release of catecholamines from the adrenal gland(1), but instead seems to block their effects. In the present experiments, more epinephrine was required to protect HSF-treated mice than adrenalectomized or β-adrenergically blocked mice, indicating that HSF may be blocking the effects of catecholamines. Fishel *et al*(3) have proposed that HSF acts by blocking the β-receptors of the adrenergic nervous system, since they found that HSF-treated mice behave similarly to those treated with β-adrenergic blocking agents. This similarity was not only in their histamine and serotonin susceptibility, but also in the glucose metabolism changes produced by these treatments. Our data substantiate the hypothesis that HSF acts by blocking some action of catecholamines, but do not indicate whether this blockade is specifically directed to the β-receptors.

Epinephrine may exert its protective action by its vasopressive effects which might help maintain circulation in spite of the hypotension produced by histamine. Whether this effect of epinephrine is the only one concerned

in the protection of mice from histamine shock is still not known. Our observations cast doubt on the importance of the vasopressor effects of epinephrine in protection against histamine toxicity since norepinephrine was less effective than epinephrine and other vasopressor agents such as ephedrine, metaraminol and angiotensin were ineffective. Epinephrine may act directly on the capillaries to decrease the loss of blood volume which occurs after histamine challenge. It has been reported that epinephrine blocks the edema produced in rats by injection of egg white or dextran solutions(19,20). Vasoconstriction did not appear to be involved in the protecting mechanism since norepinephrine was much less effective in preventing edema than was epinephrine.

The effects of HSF on glucose metabolism, observed by various workers, may be interrelated with its effects on catecholamine inhibition and enhanced vascular permeability. The dramatic hypoglycemia in HSF-treated mice after histamine challenge must have profound effects on many tissues and this aspect of this problem should be carefully investigated.

Summary. CFW mice, hypersensitized to histamine by adrenalectomy, adrenal demedullation, treatment with histamine sensitizing factor (HSF) from *Bordetella pertussis,* or β-adrenergic blocking agents, are protected from histamine death by epinephrine or norepinephrine. Intravenous treatment with these drugs, to be maximally effective, should be given 30 to 60 seconds after histamine challenge. Mice made hypersensitive to histamine apparently die from an inability of their vascular system to overcome the hypotensive effects of histamine. In adrenalectomized or adrenal-demedullated mice, there is an insufficient production of catecholamines for protection, while in the HSF-treated or β-adrenergically blocked mice the ability of endogenous catecholamines to protect seems to be diminished or blocked. When a sufficient quantity of exogenous epinephrine is given, the blocking effect of HSF or β-adrenergic blocking agents can be overcome and the mice protected from lethal histamine shock. When mice receive large sensitizing doses of HSF, protection with epinephrine is not possible.

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