THE ALTERED REACTIVITY OF MICE AFTER INOCULATION WITH *BORDETELLA PERTUSSIS* VACCINE[1]

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CONTENTS

I. INTRODUCTION

For many years the effects of bacterial inoculations have been described chiefly in terms of the antibody response of the host. Recent investigations, however, have indicated that nonspecific changes in resistance to infection can follow the injection of various bacterial products into a suitable animal (1–3). Perhaps the most unusual and most dramatic modifications of response occur in mice injected with *Bordetella pertussis* vaccine. These animals become exceedingly susceptible to the lethal effects of histamine, serotonin, anaphylaxis, and gram-negative bacterial vaccines as well as to other agents and conditions listed in table 1. It should be noted, however, that the pertussis-inoculated mouse is not a generally debilitated animal susceptible to all forms of stress. It is no more sensitive than normal animals to the lethal effects of epinephrine (28), acute heat stress (28), methacholine (28), carbaminoylcholine (11), and 48/80 (a histamine liberator) (23, 29).

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II. SENSITIVITY TO HISTAMINE

Parfentjev and Goodline (4) were the first to report that mice injected with pertussis vaccine became more susceptible to the lethal effects of histamine. This finding has been confirmed many times, the LD_{50} of histamine in pertussis inoculated mice being $\frac{1}{30}$ to $\frac{1}{300}$ the LD_{50} of this amine in unvaccinated animals. Malkiel and Hargis (12) stated that *Brucella abortus* vaccines also increase the susceptibility of mice to histamine but negative results were obtained with this vaccine by Abernathy and Spink (23). *Bordetella parapertussis*, *Bordetella bronchiseptica*, and *Haemophilus influenzae* (30, 31) do not sensitize mice to histamine; *Escherichia coli*, *Shigella dysenteriae* (11), *Salmonella typhosa* (4, 31), *Salmonella paratyphi*, and *Neisseria catarrhalis* (31) are likewise ineffective. Rats can be sensitized to histamine (30, 32) but guinea pigs (30, 33) and rabbits (33) show no increase in susceptibility after the administration of pertussis vaccine. Procedures and conditions other than pertussis inoculation which also sensitize mice to histamine are adrenalectomy (34, 35), increasing age (36), and Sarcoma 180 (37).

TABLE 1
Situations and materials to which pertussis-inoculated mice possess an enhanced sensitivity

Increased Susceptibility	References
Histamine	4
Serotonin	5–9
Anaphylaxis	10–15
Pertussis vaccine	11, 16, 17
Fractions of *Bordetella pertussis*	17
Living organisms	3, 18–20
Gram-negative vaccines	10, 11
Endotoxins	21–23
Irradiation	24
Reduced atmospheric pressure	25
Cold stress	26
Peptone	27

A. Factors Influencing the Production of Histamine Sensitivity after Pertussis Inoculation

1. Age of mice. Kind (11) reported that the LD_{50} and (19/20 confidence limits) of histamine after pertussis inoculation were 54 (31 to 92) mg per kg for 10 to 15 g mice and 17 (12 to 23) mg per kg for 20 to 30 g animals. Munoz and Schuchardt (38) found that 3 week old mice (12 ± 1 g weight) of a particular strain were more resistant to histamine sensitization after *B. pertussis* than 4 to 6 week old mice of the same strain. Maitland *et al.* (30) mentioned the fact that, with the strain of mice used in their experiments, there was no difference in sensitization between male mice weighing 14 to 16, 18 to 22, and 25 to 28 g at the time of vaccination. As a general rule it would seem prudent to use mice weighing 14 g or over to insure maximum sensitization.

2. Sex of mice. Pittman (39) reported that female mice were more susceptible to histamine than males. The difference between the LD_{50} for the two sexes was greater (86 per cent) for pertussis-inoculated than for uninoculated animals (17 per cent). Maitland *et al.* (30) mentioned similar findings; however, the differences between the sexes were somewhat smaller than those recorded by Pittman. Fink and Rothlauf (40) found no statistical difference in the reaction of uninoculated male and female mice to histamine. However, the latter challenged their animals intravenously whereas Pittman and Maitland *et al.* used the intraperitoneal route. Following Pittman's report on sex differences in the response of pertussis-inoculated mice to histamine, most investigators in this field have employed females in their experiments. In view of recent mouse shortages, however, it should be pointed out that males will serve equally well for most purposes. In a recent publication (7), the LD_{50} of histamine base in male pertussis-inoculated Tumblebrook Farm mice was 3 mg per kg as compared with 915 mg per kg in uninoculated animals.

3. Strain of mice. It is evident from the experiments of Munoz and Schuchardt (38) that strains of mice differ greatly in the degree of histamine sensitivity which appears after pertussis inoculation. Pertussis-injected, histamine-resistant mice may still show an enhanced susceptibility to the lethal effects of serotonin (5) and anaphylaxis (36).

4. Vaccine. Vaccines prepared from phase I *B. pertussis* organisms sensitize mice to histamine; vaccines made from phase IV organisms are ineffective (10, 11, 41). Investigators have produced detectable sensitization with 0.3 billion (39), 0.5 billion (38), and 0.8 billion (30) phase I *B. pertussis* cells. The degree of sensitization can be enhanced by increasing the dose of vaccine (30, 38, 39). Resistant strains of mice, however, cannot be made histamine sensitive even when the number of cells administered is increased to toxic levels. (38). Maitland *et al.* (30) found that various *B. pertussis* vaccines differed markedly in their ability to sensitize mice to histamine. The vaccines tested by the latter had previously been used by the British Medical Research Council in extensive field trials and a description of their preparation has been published (42). These vaccines differ so much with respect to the strain of organism, medium for growth, and killing agent employed that it is difficult to ascribe variation in sensitizing potency to any one of these factors. However, Maitland *et al.* (30) stated that subsequent experiments indicated that the sensitizing ability of *B. pertussis* can be influenced by the strain, the medium on which it is grown, and the method of killing it. The latter have also characterized the histamine-sensitizing factor (HSF) (30). HSF was found in *B. pertussis* but not in *B. parapertussis*; it was inactivated at 80 C in ½ hr but only slightly affected by heating at 70 C for 1 hr; it was antigenic and neutralized by antiserum; it was destroyed when bacteria were disintegrated by shaking with glass beads or by

grinding after being freeze-dried. On the basis of these properties, Maitland *et al.* differentiated HSF from other components of *B. pertussis*, namely heat labile and heat stable toxin, hemagglutinin, capsular material, and agglutinogen. Kind (16) reported that heating at 80 C for 30 min reduced the histamine-sensitizing property and destroyed the anaphylactic- and pertussis-sensitizing properties of *B. pertussis*. However, temperatures of 80 or 100 C failed to destroy the ability of pertussis vaccine to kill mice previously sensitized with unheated vaccine. It was suggested that the sensitivity of pertussis-inoculated mice to subsequent injections of pertussis is an example of sensitivity to an endotoxin-like material rather than an example of an antigen-antibody reaction.

The relationship between the histamine-sensitizing factor and the immunizing antigen of *B. pertussis* has not been completely clarified. Pittman (43) demonstrated that good sensitizing vaccines afford good protection in mice whereas poor histamine-sensitizing vaccines have low immunizing potency. However, a vaccine heated to 37 C for 30 days in the presence of phenol lost its sensitizing ability to a greater extent than its protective capacity. Maitland *et al.* (30) found that stroma protective antigen, a fraction of *B. pertussis* originally prepared by Pillemer *et al.* (44) afforded both good protection and good histamine sensitization. It is likely that further clarification of the relationship of the various components of *B. pertussis* will come from the use of procedures recently employed by Schuchardt *et al.* (45). These investigators have tested the biologic activity of antigenic fractions of *B. pertussis* shown to be immunologically homogeneous by serum diffusion techniques. By these methods they have thus far demonstrated that agglutinogen is a specific substance distinct from the substance(s) responsible for protection, histamine sensitization, toxicity, and hemagglutination.

B. Unusual Dose-Response Curve of Histamine in Pertussis-Inoculated Mice

Although the effect of histamine in mice can be quantitated by rectal temperature measurements (46), most investigators have used death as the end point. Munoz *et al.* made the interesting observation (47) that pertussis-inoculated mice challenged with histamine succumb in greater numbers to lower doses (0.5 to 2 mg per mouse) (almost an LD_{100}) than to higher doses (4 to 8 mg per mouse) of this drug. This peculiar type of response was also demonstrated in mice made histamine sensitive by adrenalectomy, but did not occur in normal histamine-resistant animals. Ambrus *et al.* (48) showed that the greater the natural resistance of a strain of mice to histamine, the less was the protection afforded by an antihistamine. When the histamine sensitivity of mice is increased by adrenalectomy or pertussis inoculation, the protective effect of antihistamines is excellent (31, 49). These findings suggest that the toxicity of histamine in pertussis-inoculated histamine-sensitive mice and in histamine-resistant animals may be manifested through two different mechanisms (48).

III. SENSITIVITY TO SEROTONIN

Interest in the altered reactivity of pertussis-inoculated mice was recently enhanced by the observations of Munoz and Greenwald (5), Munoz (6), Kind (7), Pittman (8), and Kallos and Kallos-Deffner (9) that *B. pertussis* also sensitizes these animals to the lethal effects of 5-hydroxytryptamine (serotonin). Kind (7) found that pertussis-inoculated male mice shown to be histamine sensitive were also 40 to 50 times more susceptible to intraperitoneal challenge doses of serotonin. Munoz and Greenwald (5) reported that female mice of both histamine-sensitive and histamine-resistant strains became 20 to 30 times more susceptible to intravenous doses of serotonin after the administration of pertussis vaccine. Serotonin sensitivity appeared on the first day, reached a peak about the third to fourth day and disappeared by the twenty-fifth day after the administration of *B. pertussis*. *Salmonella typhosa* and *Erysipelothrix insidiosa*, however, failed to alter the susceptibility of mice to this drug (6).

The concomitant sensitivity of the pertussis-inoculated mouse to serotonin, histamine, and anaphylaxis suggests that serotonin plays a role in anaphylactic reactions of this species. The following observations tend to support this view: (a) lethal doses of serotonin in the mouse produce symptoms similar to those observed during anaphylaxis (6); (b) the serotonin antagonists lysergic acid diethylamide (LSD) and reserpine inhibit the *in vitro* contraction of the sensitized mouse uterus upon addition of specific antigen

(50); and (c) mouse lung not only has a relatively high concentration of serotonin but also contains the enzymes which produce and destroy this amine (51). However, Munoz (6) has pointed out that LSD and reserpine are not specific anti-serotonin drugs; in addition he was unable to protect mice from anaphylactic death with these materials.

IV. SENSITIVITY TO ANAPHYLAXIS

A. Passive Anaphylaxis

The original observation that pertussis-inoculated mice became histamine sensitive (4) led to experiments which tested the susceptibility of these animals to anaphylactic shock. Kind (10, 11) reported that mice injected with *B. pertussis* vaccine possessed an enhanced sensitivity to the lethal effects of passive anaphylaxis induced by the intravenous injection of antiserum and antigen in rapid succession. Similar results were obtained by others (13, 14). Pittman (14) demonstrated that anaphylaxis occurred when an antiserum was administered either 48, 24, or 6 hr before the antigen; Munoz *et al.* (13) stated that the optimal time for challenge was 4 to 6 hr after the administration of antiserum. Both groups of investigators injected the antiserum 5 days after the mice were inoculated with *B. pertussis* vaccine (at which time the animals were maximally histamine sensitive) and Munoz *et al.* noted that it was difficult to produce sensitization after the tenth day. Pittman (14) found that 4 to 8 mg per kg of antibody nitrogen would sensitize 50 per cent of the pertussis-inoculated mice and Munoz *et al.* (13) reported that 187 μg of antibody nitrogen (9.3 mg per kg per 20 g mouse) produced consistent sensitization. Recently, Munoz *et al.* (15) have presented a detailed analysis of optimal conditions for the production of passive anaphylaxis in pertussis-injected mice.

B. Active Anaphylaxis

Malkiel and Hargis (12) demonstrated that pertussis vaccine administered along with an antigen (horse serum) sensitized mice to subsequent injections of that antigen. Of 102 mice injected intraperitoneally with pertussis vaccine and horse serum, 95 succumbed to challenge doses of horse serum administered 15 days later. In contrast, the shocking dose of horse serum killed only 3 of 66 mice previously sensitized with horse serum alone. Sensitization was induced if the horse serum was given subcutaneously and the pertussis vaccine was administered intra-abdominally; if the routes were reversed, however, no sensitization occurred (52). Likewise, if the pertussis vaccine and horse serum were injected 4 days apart the mice did not become susceptible to subsequent inoculations of horse serum. Malkiel *et al.* (52) also performed experiments to determine whether pertussis vaccine sensitized mice to active anaphylaxis by acting as an adjuvant. Crystalline egg albumin (Ea) mixed with pertussis vaccine was injected intra-abdominally into mice. A large percentage of the animals subsequently succumbed to challenge doses of the homologous antigen. However, neither precipitating, nonprecipitating, nor univalent antibodies were found in mouse anti-Ea serum. Recently, Kind (53) showed that pertussis vaccine administered to mice along with chicken red blood cells (CRBC) enhanced the production of agglutinins to CRBC and also induced anaphylactic sensitivity to subsequent challenge doses of CRBC. The ability of *B. pertussis* to stimulate the formation of agglutinins seemed to bear no relationship to its anaphylaxis-sensitizing effect. However, it is conceivable that pertussis vaccine may have augmented the manufacture of some type of sensitizing antibody which could not be measured by the methods employed. Greenberg and Fleming (54) had previously reported that pertussis vaccine enhanced the immunizing efficiency of diphtheria toxoid in guinea pigs (as measured by a change in the Schick test from positive to negative). The mechanism of the adjuvant effect of *B. pertussis* is unknown. However, it is of interest to note that Parfentjev and Manuelidis (55) found that mice injected with pertussis vaccine developed an increase in splenic weight as well as a lymphocytosis. Fichtelius and Hassler (56) inoculated adrenalectomized rats for 14 days with repeated injections of pertussis vaccine. At the end of this period the formation of lymphocytes in thymus and lymph nodes was investigated with the aid of P^{32}. Compared with adrenalectomized controls, the formation of lymphocytes was clearly increased in the pertussis animals.

The concomitant sensitivity of the pertussis-inoculated mouse to serotonin, histamine, and anaphylaxis can hardly be considered coincidental. It is tempting to postulate that enhanced susceptibility to active and passive anaphylaxis

can be explained on the basis of the concurrent sensitivity to histamine and serotonin. Active anaphylaxis, however, will occur in pertussis-inoculated mice at a time when histamine and serotonin sensitivity have declined greatly or have completely disappeared (13). Persistence of anaphylactic sensitivity in the absence of serotonin and histamine susceptibility (*e.g.*, 40 days after pertussis and antigen inoculation) can perhaps be attributed to the adjuvant properties of *B. pertussis* (53). Sufficient antibody may be available at this time to cause the release of amounts of histamine and serotonin large enough to be lethal to animals no longer sensitive to these materials. It has been demonstrated that the use of Freund's adjuvant along with the sensitizing antigen in mice results in a heightened mortality rate upon subsequent injection of the homologous antigen (57). Although the adjuvant effect of *B. pertussis* may provide an explanation for the increased susceptibility of mice to active anaphylaxis in the absence of histamine or serotonin sensitivity, the proponents of a major role of histamine and serotonin in mouse anaphylaxis may find it more difficult to account for the occurrence of enhanced sensitivity to passive anaphylaxis in the absence of histamine or serotonin sensitivity. Munoz *et al.* (15) have recently reported that CF_1 mice, which do not become histamine sensitive after pertussis vaccination, do become sensitive to passive anaphylaxis. In addition, they demonstrated that another strain of mice became sensitive to both histamine and passive anaphylaxis 5 days after pertussis inoculation; however, 7 days later, the animals were still sensitive to histamine but were no longer susceptible to passive anaphylaxis. Munoz *et al.* (15) have stated that, just as in the case of hypersensitivity to histamine, active anaphylaxis can be produced at a time when serotonin hypersensitivity has disappeared, and that the increased sensitivity to passive anaphylaxis disappears at a time when serotonin sensitivity is still present. It should be noted that mast cells contain both serotonin and histamine and that locally injected antigen initiates a more widespread degranulation of local tissue mast cells in sensitized mice than in controls (58).

Other conditions which sensitize mice to anaphylaxis are adrenalectomy (59) and ionizing radiation (60, 61). Sublethal anaphylaxis in the pertussis-inoculated mouse has been quantitated by measurements of rectal temperature drops (61, 62) and by hematocrit changes (63). The shock organ in the mouse has not been determined, although emboli have been observed in the blood vessels of the lung shortly after the administration of challenge doses of antigen (64). Recent experiments implicating serotonin in mouse anaphylaxis (5–7) suggest that the aforementioned pulmonary emboli may have been aggregates of platelets.

Although pertussis vaccine increases the susceptibility of mice to anaphylactic shock it should be noted that it does not enhance Arthus sensitivity in these animals (65) nor does it intensify the reaction of the sensitized mouse uterus to challenge doses of the homologous antigen (50). The anaphylaxis sensitizing factor of *B. pertussis* was destroyed by heating the vaccine at 80 C for 30 min (16) and various fractions of *B. pertussis* were found to possess this factor (52).

V. PERTUSSIS VACCINE AND THE ADRENAL GLAND

Observations that both adrenalectomized and pertussis-inoculated mice were more susceptible to the lethal effects of histamine and anaphylaxis than normal controls suggested that pertussis vaccine exerted its effect through injury of the adrenal gland (11). This supposition was supported by the fact that cortisone protected mice from the fatal effects of histamine (66, 67) and anaphylactic shock (68). However, Kind and Gadsden (69), Chedid (22), and Malkiel (70) presented evidence of normal adrenal function in pertussis-inoculated mice and the latter (70) also reported the absence of histological changes in the adrenal glands of such animals. Gauthier *et al.* (71) found a strain of mice which became histamine sensitive after inoculation with pertussis vaccine but not after adrenalectomy. It thus seems likely that the histamine sensitivity induced by pertussis vaccine is not due to any harmful effect of *B. pertussis* on the adrenals. The protective effect of cortisone in histamine shock and anaphylaxis may well be pharmacological in nature rather than a form of replacement therapy (71). In a recent paper, Munoz *et al.* (15) have suggested that *B. pertussis* may act at a level beyond the adrenal glands by either interfering with the utilization of steroids, enhancing their destruction, or increasing the tissue requirements for these compounds.

VI. ADDITIONAL BIOLOGICAL EFFECTS OF *B. PERTUSSIS*

Although *B. pertussis* is unique in sensitizing mice to histamine, serotonin, anaphylaxis, etc., it shares with other gram-negative organisms in the ability to alter the susceptibility of mice to bacterial infections. Landy (72) reported that lipopolysaccharides extracted from various bacteria (including *B. pertussis*) increased the resistance of mice challenged 24 hr later with *Salmonella typhosa* and also caused an elevation in properdin titer of 50 per cent or more above normal. Dubos and Schaedler (3) found that mice inoculated with *B. pertussis* or *Klebsiella pneumoniae* possessed an increased susceptibility to virulent staphylococci when infected a few hours after the administration of the vaccine. In contrast, mice infected a few days after vaccination developed an increased resistance to the challenge doses of staphylococci. Dubos and Schaedler (3) likewise presented excellent arguments against the role of properdin in the nonspecific resistance of mice to bacterial infections.

Although *B. pertussis* as well as other organisms may induce an enhanced resistance of mice to bacterial infections, *B. pertussis* also has the unique property of lowering the resistance of mice to living heterologous organisms (at a time when elevated properdin titers would be expected). Parfentjev *et al.* reported that *B. pertussis* increased the susceptibility of mice to challenge doses of *Proteus vulgaris* (18), *Pasteurella multocida* (18), and *Pseudomonas fluorescens* (20) (at a time when Dubos and Schaedler (3) demonstrated increased heterologous resistance). The author (unpublished observations) has recently found that, 13 days after pertussis inoculation, mice possessed an enhanced susceptibility to the lethal effects of *Escherichia coli* endotoxin as well as to the intraperitoneal injection of 2.3×10^6 living *E. coli* organisms. The large number of organisms needed to produce a fatal outcome (as was the case in some of the aforementioned experiments of Parfentjev *et al.*), as well as the concomitant sensitivity to endotoxin, suggest that the cause of death was endotoxic in nature.

B. pertussis is a complex organism. It contains, among many other components, a lipopolysaccharide which may cause an enhanced heterologous resistance to bacterial infection; it likewise possesses a factor which sensitizes mice to endotoxin, thus causing an increased susceptibility to infection with organisms whose virulence may to some extent be due to their endotoxin content. Whether pertussis inoculation will result in enhanced resistance or enhanced susceptibility will thus likely depend upon such factors as the dose of pertussis vaccine administered and the nature, virulence, and route of inoculation of the infecting organism.

Andersen (73) has confirmed the observations of Evans and Perkins (74) that pertussis vaccine given simultaneously (intracerebrally) with the challenge dose of *B. pertussis* was able to protect against death. The protective effect of pertussis vaccine was shown to have both nonspecific and specific components. Andersen reported (75) that pertussis vaccine caused a decrease in resistance to heterologous infection during the first 2 weeks and an increased resistance 20 to 30 days after infection. The latter also found (76) that intranasal inoculation of *B. pertussis* in mice produced pulmonary edema and that sterile extracts from virulent strains had the same effect. Pittman (77) had previously demonstrated that respiratory infection with *B. pertussis* sensitized mice to histamine and that the development of histamine sensitivity was correlated with the presence of lung lesions. The ability of pertussis vaccine to induce so many biological changes in the mouse not only explains difficulties in obtaining reproducible results in mouse protection tests with *B. pertussis* but also indicates the necessity for caution in the interpretation of such tests.

VII. HYPOTHESES

Since pertussis-inoculated mice possess an enhanced sensitivity to the lethal effects of histamine and serotonin it is tempting to postulate that the increased susceptibility of these animals to anaphylaxis, peptone, endotoxins, etc., is owing to the release of histamine and serotonin by these agents and conditions. Experiments designed to test this hypothesis have been difficult to evaluate. Thus, Hunder and Spink (27) found that mice inoculated with pertussis vaccine were sensitized to the lethal effects of histamine and peptone. Such animals could be protected from peptone shock with an antihistamine. Paradoxically, however, a strain of mice which does not become histamine sensitive after pertussis inoculation also developed a susceptibility to the lethal effects of peptone. The failure of antihistamines to protect pertussis-inoculated mice from endo-

toxic death has been attributed to the possible inability of antihistamines to reach the site of action of endogenously released histamine (23). On the other hand, interpretations based on positive protective effects of antihistamines and antiserotonin drugs have been questioned on the basis of a presumed lack of specificity of these agents (6, 60). Experimental data listing serotonin and histamine values in the organs, blood, and plasma of pertussis-inoculated mice during anaphylactic, peptone, and endotoxic shock would be most valuable.

The manner in which pertussis vaccine alters the mouse so that it becomes so highly sensitive to histamine and serotonin has not yet been determined. It may be that *B. pertussis* interferes with the destruction of these amines by inhibition of monoamine and diamine oxidase. Kind and Woods (78) reported that the lungs of pertussis-inoculated mice had a reduced histaminase activity; however, these findings could not be reproduced by the authors in subsequent experiments. Angelakos and Loew (79) were unable to increase the histamine toxicity in mice and rats following treatment with histaminase inhibitors (with the possible exception of imidazole). Fishel (80) could not correlate the decreased resistance of the pertussis-injected mouse to histamine with specific alteration in the free amino acid concentration of various tissues. Likewise no increase in the histamine content of tissues could be found following pertussis inoculation.

Present investigations of the unique sensitizing properties of *B. pertussis* are likely based on the two following assumptions: (a) sensitivity to histamine, serotonin, anaphylaxis, endotoxins, etc., is due to a defect in steroid metabolism and (b) increased susceptibility to the lethal effects of endotoxins, anaphylaxis, peptones, etc., is due to the release of histamine and/or serotonin in an animal somehow made sensitive to histamine and serotonin. The first proposition would seem to be amenable to proof or disproof in the near future. The second assumption is probably an oversimplification and its clarification will depend upon a better understanding of the complex interrelationships between histamine, serotonin, epinephrine, and acetylcholine. In this connection, studies of mast cells (which contain both serotonin and histamine) will certainly bear watching.

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