

RELATIONSHIP OF INCREASED HISTIDINE DECARBOXYLASE ACTIVITY TO BORDETELLA PERTUSSIS VACCINE SENSITIZATION OF MICE

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Bordetella pertussis vaccine is capable of rendering certain strains of mice highly sensitive to histamine and serotonin, to anaphylactic shock, and to a number of other agents and conditions.¹

In this paper it is shown that injection of mice with pertussis vaccine increases histidine decarboxylase activity in certain tissues. Evidence is presented which suggests that this biochemical change is related to the phenomenon of sensitization.

Histamine is mobilized in mammals by two principal processes: one is by rapid release from the bound condition; the second process is by new synthesis of histamine catalyzed by an adaptive form of histidine decarboxylase.* This enzyme is activated by a number of different stimuli.²⁻⁶

Our findings lead to the hypothesis that pertussis sensitization is due to initiation and maintenance of new histamine synthesis in or near sensitive cells of mouse lung, and possibly of other tissues, without concomitant increase in adrenal steroid output. The continued exposure of these cells to newly synthesized histamine may produce a reversible alteration of the cell which can be rendered irreversible and lethal by injection or release of histamine or serotonin.

MATERIALS AND METHODS

Determination of Histidine Decarboxylase Activity.—The method in detail has been published.⁵ Briefly, it consists of breaking tissue cells by repeated freezing and thawing, then centrifugation at high speed. Aliquots of the supernate are incubated with 10 γ C¹⁴L-histidine for three hours at 37° C. under nitrogen. C¹⁴ histamine formed is determined by isotope dilution assay; non-isotopic carrier histamine is added, extracted, converted to dibenzenesulfonyl-histamine (BSH), recrystallized, and counted in a liquid scintillation counter. Results are calculated as counts per minute per 100 mg. BSH.

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*It has been shown by two techniques that there is a correspondence between in vitro histidine decarboxylase assays and in vivo histamine synthesis.^{4,6} Histamine formed by inducible histidine decarboxylase is called induced histamine to distinguish it from the bound histamine which may be present in many tissues.

Blank values generally are 30 to 50 units, control lungs 20 to 60 units after subtraction of blank, and lungs of pertussis-treated mice generally three to eight times the values of the controls. In this paper, for comparison of experiments done at different times and for ease in evaluation of percentage increases of enzyme activity, all results were recalculated so that the average of the control samples arbitrarily equals 100.

Determination of Blood Corticosterone Levels.—The method of Silber, Busch, and Oslapas⁷ was used.*

B. PERTUSSIS Sensitization.—Mice (Tumblebrook strain and CF₁ strain) were sensitized to serotonin or histamine toxicity by the intraperitoneal injection of 2 billion smooth killed *B. pertussis* cells. After a suitable interval as indicated, sensitivity was measured by the administration of 10 mg. per kilogram of serotonin intravenously or 25 mg. per kilogram of histamine intraperitoneally. In anaphylaxis studies, the *B. pertussis*-treated mice were passively immunized to crystalline egg albumin by the intraperitoneal injection of 0.15 to 0.2 ml. of mouse anti-egg albumin ascitic fluid (250 γ antibody nitrogen per milliliter), and one hour thereafter were challenged intravenously with 0.5 mg. of commercial crystalline egg albumin in 0.2 ml. saline. When histidine decarboxylase activity was assayed, mice were killed three or four days after pertussis injection and the pooled tissues of several mice, usually three, assayed.

Staphylococcus Vaccine.—*Staphylococcus aureus* (Smith strain) was grown for twenty-four hours in brain heart broth and then autoclaved for fifteen minutes and diluted 2 parts vaccine with 1 part of saline. Mice were injected intraperitoneally with 0.25 ml.

Typhoid Vaccine.—Combination typhoid-paratyphoid vaccine was purchased from Lederle Laboratories. Mice were injected intraperitoneally with 0.25 ml.

Proteus Vaccine.—*Proteus vulgaris* strain 1810 was grown for eighteen hours in brain heart broth and, after the addition of formalin (final concentration 1 per cent), incubated for three days at 37° C. Mice were injected intraperitoneally with 0.25 ml.

Freund's Adjuvant.—Freund's adjuvant consisted of 2 parts of White Oil N.F. containing 2 mg. per milliliter *Mycobacterium phlei*, 1 part Arlacel A.†

RESULTS

Effect of B. PERTUSSIS Vaccine on Histidine Decarboxylase Activity of Mouse Lung.—The original observation that treatment of mice with pertussis vaccine results in increased lung histidine decarboxylase activity three days after injection has been reported.² Results for TF and CF mice are shown in Experiments A and B, Table I.

*The authors are indebted to Dr. R. H. Silber and Mr. Paul Arcese for the corticosterone assays.

†Atlas Powder Co., Wilmington, Delaware.

TABLE I. EFFECT OF VARIOUS SUBSTANCES AND CONDITIONS ON HISTIDINE DECARBOXYLASE ACTIVITY OF MOUSE LUNG

EXPER.	TREATMENT*	HISTIDINE DECARBOXYLASE ACTIVITY (RELATIVE TO CONTROLS = 100)†	AVERAGE
A	Control (TF mice‡)	58, 101, 103, 138	100
	Pertussis	310, 650, 1030, 1100	770
B	Control (CF mice)	73, 78, 117, 132	100
	Pertussis	516, 652, 698, 838	676
C	Control	54, 96, 110, 110, 130	100
	Pertussis	266, 460, 666, 1930	830
	Pertussis (heated)	168, 416, 504, 1290	595
D	Control liver	79, 91, 94, 106, 130	100
	Pertussis liver	161, 167, 179, 192	175
E	Control leg muscle	83, 90, 96, 114, 117	100
	Pertussis leg muscle	100, 129, 163, 206, 219	163
F	Control spleen	43, 96, 161	100
	Pertussis spleen	175, 408, 703	429
G	Control skin	96, 100, 104	100
	Pertussis skin	89, 107, 117	104
H	Control	84, 105, 111	100
	Staphylococcus vaccine	43, 80, 113	79
I	Control	92, 103, 105	100
	Typhoid vaccine	23, 23, 99	48
J	Control	79, 108, 113	100
	Proteus vaccine	117, 160, 188	155
K	Control	88, 93, 119	100
	Freund's adjuvant (oil only)	133, 135, 135	134
	Freund's adjuvant + <i>M. phlei</i>	560, 737, 781	693
L	Control	88, 100, 112	100
	<i>M. phlei</i> in aqueous medium	53, 76, 132	87
	Freund's adjuvant (oil only)	68, 115, 132	105
	Freund's adjuvant + <i>M. phlei</i>	438, 485, 804	576

*See text for full details.

†For explanation, see Methods.

‡TF mice used, and lungs taken for assay three or four days after injection unless otherwise noted.

Histidine Decarboxylase Activity of Mouse Lung at Various Intervals After Injection of B. PERTUSSIS Vaccine.—Mice were killed on various days following inoculation; results are shown in Fig. 1. In the first curve, one of the early experiments, maximum enzyme activity was observed on the third day. The second curve is from data obtained several months later; the maximum was obtained on the fourth day. In accordance with these findings, in the earlier experiments, animals were tested three days after pertussis injection, and, in later experiments, four days after injection.

TF mice injected with pertussis vaccine reach maximum sensitivity to passive anaphylaxis on the third or fourth day; sensitivity is reported to persist for eight to ten days.⁸ Sensitivity to injected serotonin and histamine also becomes maximal on about the third or fourth day but persists somewhat longer; sensitivity to serotonin appears to be lost between the twelfth and eighteenth day⁹ while sensitivity to histamine is reported lost between the fourteenth and twenty-eighth days.⁸ Fig. 1 shows that histidine decarboxylase activity of lung reaches a maximum on the third or fourth day, is slightly elevated on the eighth day, and at the thirteenth day is no longer detectable; no detailed exploration of the time of return of normal enzyme activity was made. Thus, there is a fundamentally good agreement between the enzyme activity curve and the periods of

sensitization. Discrepancies may be explicable by one or more of the following: (a) the *in vitro* enzyme activities may not correspond exactly to *in vivo* histamine synthesis, (b) after sensitivity of cells is established, a very low rate of histamine synthesis may be adequate for maintenance, (c) biological sensitivity may be influenced by all tissues where enzyme activation occurs; the enzyme time curve was determined for lung only, (d) histamine and serotonin may act primarily on different tissues, (e) the exact time when sensitization is lost may be subject to more precise evaluation, (f) enzyme activities and sensitivity studies were done under different conditions.

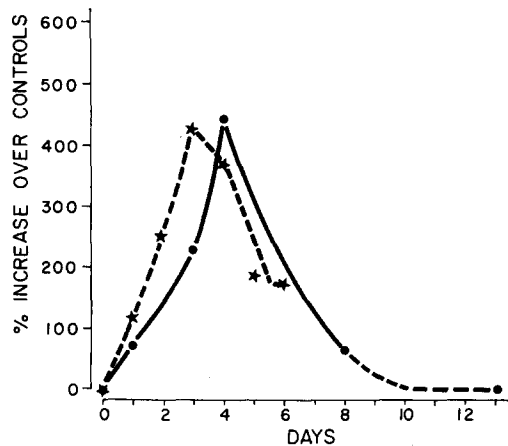


Fig. 1.—Curves of two experiments showing histidine decarboxylase activity of mouse lung at various intervals after injection of *B. pertussis* vaccine.

Effect of Pertussis Vaccine on Corticosterone Levels of Mouse Blood.—Table II shows that treatment of mice with pertussis vaccine results in a rapid increase in the blood concentration of the principal adrenal steroid of this species, corticosterone; the levels then return to normal at some time between the seventh and sixteenth hours. This early rise is probably an endotoxin effect.* Comparison of the data of Table II with those of Fig. 1 shows that during the greater part of the period of increased histidine decarboxylase activity, there is no compensation due to increased adrenal steroid output.

Effect of Heat.—Heating pertussis vaccine at 56° C. for thirty minutes inactivates a toxic ingredient but does not destroy the sensitizing factor.¹⁰ The data of Experiment C (Table I) show that heated vaccine is capable of increasing histidine decarboxylase activity of mouse lung.

Effect of Pertussis Vaccine on Histidine Decarboxylase Activity of Other Mouse Tissues.—Under conditions in which a sixfold increase in lung histidine

*Pertussis vaccine contains endotoxin; like other endotoxins it causes a rapid increase in histidine decarboxylase activity which reaches a maximum in about six hours and returns essentially to normal in a day or less.^{4,6} The vaccines of typhoid, proteus, and staphylococcus show this rapid effect also. It is a typical systemic stress reaction; the time of increased histidine decarboxylase activity corresponds closely to the time of increased corticosterone output. Hence neither the histamine nor the adrenal steroid is markedly excessive during this period. It is the protracted increase of histamine production, which becomes uncompensated at some time between the seventh and sixteenth hour, which is believed to cause sensitization.

TABLE II. CORTICOSTERONE LEVELS OF MOUSE SERUM AT VARIOUS INTERVALS AFTER INJECTION OF *B. pertussis* VACCINE

TIME (HR.)	CORTICOSTERONE (γ /ML.)	
	EXPER. I	EXPER. II
0 (Control)	0.32	0.34
0.5	0.44	0.42
2	0.66	0.58
4	0.50	0.58
7	0.40	0.44
16	0.24	0.20
24	0.14	0.30
48	0.21	0.30
72	0.12	0.12
96	0.22	0.26

decarboxylase was obtained, liver and leg muscle from the same mice showed only a slight increase in enzyme activity. Values for spleen were elevated although quite variable; skin was unaffected (Experiments D, E, F, and G).

Absence of Prolonged Activation of Histidine Decarboxylase by Various Other Bacterial Products.—Endotoxins produce a rapid increase in histidine decarboxylase activity which reaches a maximum in about six hours. Thereafter it returns essentially to normal in one day⁴ and is not elevated on the third or fourth day after injection. *Mycobacterium phlei* in aqueous medium, and typhoid, proteus, and staphylococcus vaccines had no significant effect on enzyme activity of mouse lung three or four days after injection (Table I, Experiments H, I, J, and L). Thus, the prolonged activating effect of pertussis vaccine on the enzyme is not shared by some other bacterial products.

Studies on Freund's Adjuvant.—If another substance were to be found which induced a prolonged increase in mouse lung histidine decarboxylase and also produced sensitization, the hypothesis would be materially strengthened. Freund's adjuvant containing killed *Mycobacterium phlei* was tested because, like pertussis vaccine, it has biological effects which seem to be independent of the adjuvant action. *M. phlei*, Freund's adjuvant without *M. phlei*, and complete Freund's adjuvant were injected intraperitoneally in 0.25 ml. quantities. Results of histidine decarboxylase assays are shown in Experiments K and L, Table I. Only the Freund's adjuvant containing killed *M. phlei* produced an increase in enzyme activity.

TABLE III. CORTICOSTERONE LEVELS OF MOUSE SERUM AT VARIOUS INTERVALS AFTER INJECTION OF COMPLETE FREUND'S ADJUVANT

TIME (HR.)	CORTICOSTERONE (γ /ML.)	
	EXPER. I	EXPER. II
0 (Control)	0.14	0.20
0.5	0.66	0.52
2	0.56	0.58
4	0.72	0.60
7	0.38	0.36
16	0.36	0.50
24	0.52	0.30
48	0.46	0.42
72	0.26	0.32

Since complete Freund's adjuvant produced a sustained increase of histidine decarboxylase activity in mouse lung, the effect of the adjuvant on sensitization of mice was tested. Mice were injected intraperitoneally with 0.25 ml. of the adjuvant and were challenged three days later with 75 mg. per kilogram serotonin base intravenously. There was no evidence of sensitization; all mice survived. In this case, mice showed increased histidine decarboxylase activity of the lung but were not sensitized.

To maintain the hypothesis that sensitivity is due to the induced enzyme, it was necessary to obtain evidence for adrenal stimulation other than the obviously stressed appearance of Freund's adjuvant-treated mice. Table IV shows that blood corticosterone levels of Freund's-treated mice are rapidly increased and that the augmented production is still present three days after injection. Thus, Freund's adjuvant probably does not sensitize because it stimulates a large output of adrenal steroids.

TABLE IV. EFFECT OF HYDROCORTISONE ON SENSITIZATION WITH *B. pertussis* VACCINE

COMPOUND	TIME BETWEEN CESSATION OF HYDROCORTISONE TREATMENT AND CHALLENGE (HR.)	SURVIVAL/TOTAL	PER CENT SURVIVAL
Control	24	8/40	20
Hydrocortisone		27/35	77
Control	48	6/30	20
Hydrocortisone		21/29	72
Control	72	7/29	24
Hydrocortisone		11/29	37
Control	96	10/29	34
Hydrocortisone		9/26	34

Effect of Hydrocortisone on Sensitization.—If prolonged exposure of inadequately protected cells to newly synthesized histamine is the cause of sensitization, then protection of these cells from the effects of histamine should prevent sensitization. Mice were given a diet containing 60 mg. per kilogram of hydrocortisone starting one day before *B. pertussis* injection and for three days thereafter. Hydrocortisone is completely eliminated from the body of the mouse within a few hours after ingestion.* The mice were then challenged with serotonin at various intervals after cessation of hydrocortisone intake. The data of Table IV show that mice are protected from death for forty-eight hours after cessation of treatment with hydrocortisone. The sensitivity then returns, becoming essentially complete in three days. These results are in excellent agreement with those predicted on the basis of the hypothesis.†

*Injected corticosterone, and corticosterone synthesized endogenously after injection of the precursor, are virtually undetectable in mice within two hours.²⁹ Hydrocortisone behaves similarly (Darrach, M., personal communication). The gastrointestinal tract of the mouse empties in two to six hours after feeding (Bosshardt, D., unpublished data). Thus about eight hours is the maximum time ingested hydrocortisone might be expected to remain in the body. This conclusion has been confirmed by feeding C¹⁴ hydrocortisone to mice (Ganley, O. H., unpublished data).

†In rats, adrenal steroids suppress histidine decarboxylase activity of lung³⁰; this effect has not been detected in mice (Schayer, R. W., unpublished data) although several steroids have been tested. It is, therefore, unlikely that inhibition of pertussis sensitization by hydrocortisone is related to suppression of histidine decarboxylase activity.

DISCUSSION

It is our belief that the sensitizing properties of pertussis vaccine in the mouse are due to its ability to initiate a prolonged increase in the rate of histamine synthesis in lung, and possibly other tissues, without a concomitant increase in adrenal steroid output. Because of the long time required for each assay, lung is the only tissue studied extensively. Other tissues may also participate in sensitization* and may differ from lung in time of maximal enzyme activity and in length of activation. The results on lung are considered as an example of the basic mechanism leading to sensitization and suggest that lung may be an important shock tissue, but not necessarily the only one.

The results of all of our experiments and the results of many experiments reported by other laboratories are compatible with the hypothesis stated above.

Kind¹ has listed the compounds to which pertussis-treated mice do not show increased sensitivity. They are epinephrine, methacholine, carbaminocholine, and Compound 48/80. According to the hypothesis, epinephrine should not be harmful, because it antagonizes histamine. The choline esters are known to release epinephrine and norepinephrine from the adrenals.¹¹ Compound 48/80 disrupts mast cells and releases histamine in the rat and several other species. In the mouse, however, it is not a very effective mast cell disruptor¹² and there is evidence that the toxic effect of Compound 48/80 in mice is due to factors other than histamine release.¹³

Of the substances listed by Kind¹ other than histamine and serotonin to which pertussis-inoculated mice are susceptible, peptone may act through release of amines; all the other substances listed by Kind contain bacterial endotoxins or may have endotoxin-like properties. In view of the evidence for participation of induced histamine in endotoxin shock⁴ it would be expected that a histamine-sensitive animal would suffer a delayed death from a low dose of endotoxin; not a rapid death as in anaphylaxis. These time relationships are observed in pertussis-inoculated mice.

The hypothesis is compatible with numerous reports of differences among various strains of mice in sensitivity to histamine, serotonin, and anaphylaxis. These differences may be explicable in terms of the rate and duration of histamine synthesis in various tissues, whether such tissues are specific targets for injected histamine or serotonin or for substances released in anaphylaxis, whether adrenal steroids effectively protect the target organ, the number and location of mast cells, their content of histamine and serotonin, the susceptibility of mast cells in various locations to disruption, the ability of injected or released amines to reach the sensitized cells, the capacity of various tissues for inactivating histamine and serotonin, availability of protective compounds, such as norepinephrine, from the sympathetic nerve endings, and to numerous other factors.

*The degree of sensitization of cells is, of course, not necessarily proportional to the per cent increase of histidine decarboxylase activity in a tissue over normal values. Hence any tissue, in which a significant, prolonged increase of enzyme activity is observed, must be considered as a possible contributor to the sensitization of the mouse.

Only one other change in enzyme activity seems to have been reported in pertussis-sensitized mice. Kind and co-workers¹⁴ found a reduction of histaminase activity of mouse lung but were unable to confirm this at a later date.¹ Niwa and co-authors¹⁵ published similar findings in tissues of pertussis-treated mice and rats. It is doubtful that a drop in histaminase activity could be of significance in sensitization. Histaminase is of minor importance in histamine destruction in mice; methylation is the principal means of inactivation in this species.¹⁶ Furthermore, mice are sensitized to serotonin which is not a substrate of histaminase.

The failure of Fishel¹⁷ to find increased histamine concentrations in tissues of sensitized mice does not oppose the hypothesis. The concentration of "bound" histamine, which is protected from destruction, may be quite high in some tissues. Induced histamine is not bound and is rapidly destroyed. Hence an increased rate of synthesis of the latter might not produce a significant rise in tissue histamine. The efficacy of the newly synthesized histamine may lie not in quantity but in site of formation and length of exposure of cells to it.⁴

Pertussis vaccine seems to contain one or more substances which is retained in lung, probably in reticuloendothelial cells¹⁸; like other activators of the reticuloendothelial system tested, it also activates histidine decarboxylase.^{4, 5} The period of retention of the stimulant in these cells may be reflected by the duration of increased enzyme activity.

In pertussis-vaccinated mice, certain histamine-sensitive cells, such as those of smooth muscle and endothelium, may undergo changes due to a prolonged state of abnormal permeability of the membrane. Eyring and Dougherty¹⁹ have discussed the nature and significance of the permeable state, the dangers to the cell of remaining in the permeable state for prolonged periods, and reasons for implicating histamine in permeability changes.

As a result of its initiation and maintenance of uncompensated histamine synthesis, pertussis vaccine presumably increases the need of certain tissues for adrenal steroids to restore the normal permeability. This state could account for the observations that pertussis-vaccinated mice, despite evidence of normal adrenal function,²⁰⁻²² behave in many respects like adrenalectomized mice.²³ Adrenalectomy renders mice histamine-sensitive by removal of histamine-antagonizing hormones²⁴; pertussis vaccine may render mice histamine-sensitive by inducing endogenous histamine synthesis and thus reducing the threshold concentration of exogenous histamine required to cause irreversible changes.

Thus, the hypothesis on the mechanism of pertussis sensitization is compatible with the results of a large number of highly diversified experiments. Some of these, the time curve (Fig. 1) and the hydrocortisone protection experiments, have submitted the present interpretation to rigorous tests. The authors are not aware of a single well-established observation which opposes the conclusions of this paper.

Finally, the hypothesis is logical. If histamine and serotonin, the latter having histamine-like properties in rats and mice,²⁵ are unique in ability to produce damage in a pertussis-sensitized mouse by direct action, then one of

these amines, if mobilized in a slow, prolonged manner, in sublethal quantities, should also be capable of effecting the initial sensitization. Induced histamine fully satisfies these requirements; attempts to demonstrate increased serotonin production in cases in which increased histamine synthesis occurs have been unsuccessful.²⁶⁻²⁸

SUMMARY

Injection of mice with *Bordetella pertussis* vaccine, a procedure which renders certain strains of mice highly sensitive to histamine, serotonin, and anaphylactic shock, also leads to increased histidine decarboxylase activity in lung during a period which corresponds closely to the time of sensitivity.

The hypothesis is presented that pertussis sensitization is caused by prolonged exposure of certain cells of lung, and possibly of other tissues, to newly-synthesized histamine. In the absence of a significantly increased output of protective hormones from the adrenals, this histamine may produce a reversible alteration of permeability in the membranes of the affected cells and changes in the intracellular organization; then injection or release of histamine and serotonin may result in irreversible damage and death.

A number of experiments are described which are compatible with this hypothesis.

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