# Histamine Sensitivity of Adrenalectomized and Pertussis-Treated Mice. (22150)

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It has been established that mice, which are normally resistant to histamine, become highly sensitive after being injected with *Hemophilus pertussis* vaccine (see refs. in 1-10). The mechanism by which this vaccine increases the sensitivity to histamine by 25 or 100 times remains unknown, although several authors have suggested that diminished function of adrenal cortex and medulla may be involved since adrenalectomized mice are known to be sensitive to histamine (usually injected intraperitoneally) (10-14), and sensitivity is partially or completely lost after treatment with epinephrine and cortisone (5,14). It seemed pertinent to make a quantitative study to determine the degree of histamine sensitivity which develops in mice following treatment with pertussis-vaccine as compared directly with the sensitivity following adrenalectomy. The results of this study revealed that the sensitivity to histamine induced with pertussis vaccine could scarcely be related to altered function of the adrenal gland since adrenalectomized mice of the strain used were no more sensitive to intravenous injections of histamine than normal mice, whereas pertussis treatment of intact mice enhanced sensitivity to histamine 33 times.

*Methods.* The general plan of the experiment was to determine concurrently the $LD_{50}$ of histamine diphosphate in normal, adrenalectomized and pertussis-treated mice in order to make a direct comparison of histamine sensitivity in the 3 types of animals. Female albino mice of the Swiss-Webster strain (Taconic) weighing between 15 and 27 g were obtained from a local source, and maintained before and after operation or vaccine injection on a diet of Purina Laboratory Chow. Bilateral adrenalectomies were conducted under light ether anesthesia and operated mice were provided with 0.9% saline until injected with histamine the following day. All other mice were supplied with tap water *ad lib.* *Hemophilus pertussis* vaccine supplied by the Massachusetts State Health Department contained 20 billion Phase I organisms per ml and Merthiolate in concentration of 1:10,000. This was diluted with saline so that each 0.5 ml contained 8.75 billion organisms, this volume being injected intraperitoneally in mice 4 days prior to histamine injection. Mice in control groups were intact and untreated at the time of histamine injection. Histamine diphosphate solutions were prepared in normal saline, then several dilutions in saline were made to obtain concentrations which permitted injection of various doses (Table I) in constant volume of 0.01 ml per gram of mouse. Histamine solutions were refrigerated and used within 48 hours of preparation. Injections of histamine were made into the tail vein during a period of 10 seconds, 4 graded doses being injected into normal, adrenalectomized and pertussis-treated mice on each day of experimentation. Each dose of histamine was injected into 20 mice, with exceptions as indicated in Table I.

Completeness of adrenalectomy was checked by providing a few mice from each operated group with 0.9% saline for 24 hr and tap water thereafter, incidence of mortality being recorded during 14 days. Some of these mice were fed the Purina Chow, others were fed oatmeal which has a lower sodium content. Histamine injections were made in adrenalectomized mice 24 hr after operation in order to minimize the possibility of growth of adrenal rests. Incidence of mortality was recorded as the number of mice dead per total number injected for a given dosage level after 24 hours. $LD_{50}$ values were computed according to the method of Litchfield and Wilcoxon(15).

TABLE I. Acute Toxicity of Histamine Diphosphate in Normal, Adrenalectomized and Pertussis-Treated Mice as a Measure of Histamine Sensitivity.

| Treatment of mice | Histamine diphosphate, mg/kg, I.V. | Mortality | | $LD_{50}$, mg/kg, I.V. (fiducial limits) 95% |
|---|---|---|---|---|
| | | Ratio | % | |
| Normal (controls) | 300 | 4/20 | 20 | 333 |
| | 350 | 9/20 | 45 | (309-367) |
| | 400 | 16/20 | 80 | |
| | 550 | 16/16 | 100 | |
| Adrenalectomized | 150 | 0/ 6 | 0 | 315 |
| | 250 | 1/20 | 5 | (284-350) |
| | 300 | 12/20 | 60 | |
| | 350 | 13/20 | 65 | |
| | 400 | 15/20 | 75 | |
| Pertussis-treated | 6 | 2/20 | 10 | 10 |
| | 8 | 8/20 | 40 | (8.8-12.3) |
| | 10 | 8/20 | 40 | |
| | 14 | 14/20 | 70 | |

*Results.* Data presented in Table I reveal that pertussis-treated mice were 33 times more sensitive than normal mice to histamine injected intravenously, as indicated by $LD_{50}$ values of 10 and 333 mg/kg, respectively. However, the adrenalectomized mice were no more sensitive than normal mice to histamine injected intravenously, since the $LD_{50}$ values of 315 and 333 mg/kg, respectively, are almost identical.

Failure of adrenalectomized mice to exhibit sensitivity to histamine injected intravenously could not be due to incomplete adrenalectomy or growth of adrenal rests. A majority of the mice were considered to be completely adrenalectomized because: a. 63% of 71 adrenalectomized mice provided with Purina Chow and tap water died in 14 days; 94% of 16 adrenalectomized mice died in 14 days when provided with tap water and oatmeal, the latter having a lower sodium content than Purina Chow. b. Histamine was purposely injected 24 hr after adrenalectomy, thus eliminating the possibility of significant growth of adrenal rests. c. Furthermore, an experiment was made which confirmed the reports that adrenalectomized mice become sensitive to histamine injected intraperitoneally(10-14). Intraperitoneal injection of graded doses of histamine in 40 adrenalectomized mice revealed that they were 3 to 7 times as sensitive as normal mice.

*Discussion.* The evidence presented clearly indicates that pertussis-treatment greatly increased sensitivity to histamine in the strain of mice used. It is clear that this strain of mice did not become sensitive to histamine injected intravenously after complete adrenalectomy. The failure of these adrenalectomized mice to exhibit sensitivity to histamine is not a unique finding since a CF-1 strain of mice was shown not to be sensitive after either injections of pertussis vaccine(10) or adrenalectomy(13). The important point indicated by the data herein presented is that the mice became sensitive to histamine after injection of pertussis vaccine, but not after adrenalectomy. This experiment therefore provides definite evidence that sensitivity to histamine which developed after treatment with pertussis vaccine cannot be ascribed to diminished function of the adrenal cortex and/or medulla, or to diminished action of hormones from the adrenal gland.

Although several authors have suggested that histamine sensitivity induced by pertussis vaccine is due to an injurious action of the vaccine on the adrenal glands, there has been no direct evidence presented to support the belief. If pertussis vaccine diminished the function of the adrenal medulla and cortex in mice one would expect the animals to become susceptible to various forms of stress. However, Kind(4) states that pertussis-treated mice did not become sensitive to injections of methacholine. Furthermore, the susceptibility to severe hypoxia(6) was accompanied by liver glycogen values which were not in-

dicative of abnormal adrenal cortical function. No evidence was presented to implicate abnormal adrenal function as the cause of hypoglycemia which developed in pertussis-vaccine treated mice(2). Other evidence exists which suggests the possibility that pertussis vaccine causes dysfunction of the adrenal glands. The sensitivity to histamine (injected I.P.) in pertussis-treated and adrenalectomized mice is partially or completely restored by proper treatment with cortisone (5) or a combination of cortisone and epinephrine(14). Nevertheless, the effects of these substances may be pharmacological in nature rather than specific physiological effects of replacement therapy after adrenal inactivation.

The authors are not aware of published data which prove that pertussis-vaccine alters the adrenal cortex as indicated by cholesterol content, ascorbic acid depletion, or other tests of adrenal function.

*Summary.* In a Swiss-Webster strain of mice, pertussis vaccine treatment increased the sensitivity to intravenously injected histamine 33 times, whereas surgical adrenalectomy had no significant effect on sensitivity to histamine. This finding, in addition to lack of direct evidence to the contrary, warrants the conclusion that *Hemophilus pertussis* vaccine enhances sensitivity of mice to histamine through some mechanism other than impairment of adrenal function.

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Received November 21, 1955. P.S.E.B.M., 1955, v90.

## Diet and TEPA Therapy in Sarcoma-Bearing Rats.* (22151)

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Data have been presented to demonstrate that N, N', N'' triethylenephosphoramide (TEPA) reduced food efficiency and growth of normal as well as tumor tissue in tumor-bearing rats(1). Given an optimum dosage and a susceptible tumor, however, reduction in growth of the tumor was greater than reduction in weight of normal tissues(2). These data also demonstrated that a high protein diet, or a low casein diet supplemented with methionine, reduced the depleting effect of TEPA on normal tissue without altering the deleterious effect on the tumor(1,2). Other experiments have demonstrated that supplementing a low casein diet with a mixture of methionine and guanidoacetic acid or methionine and glycine resulted in increased protection of normal tissues to the depleting effects of the tumor(3). Further work has been done to determine the effect of these mixed supplementations upon chemotherapy of TEPA in tumor-bearing rats.

* Some of these results were read before the Am. Chem. Soc. Meeting in New York, Sept. 1954. These studies were supported by an Institutional Grant from the American Cancer Society.