INHIBITION OF HISTAMINE DEATH IN PERTUSSIS-INOCULATED MICE BY CORTISONE AND NEOANTERGAN

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THE guinea pig is naturally sensitive to histamine; for this reason, it has been widely employed in the screening of compounds which inhibit the effects of this toxic amine. The mouse is naturally resistant to histamine; however, Parfentjev and Goodline have shown that previous inoculation of mice with Hemophilus pertussis vaccine greatly increases the sensitivity of these animals to histamine. In an experiment performed by the author, the LD₅₀ of histamine in uninoculated mice was found to be 2300 mg./kg, whereas the LD₅₀ of histamine in pertussis-inoculated mice was only 23 mg./kg. This report demonstrates that the antihistamine, Neoantergan, and the adrenal steroid 11-dehydro-17-hydroxy corticosterone (Cortone, Merck), protected pertussis-inoculated, histamine-sensitive mice from the lethal effects of histamine. Histamine death in guinea pigs has been inhibited by Neoantergan; however, cortisone and ACTH have not been effective.

Some of the following experiments also show that cortisone protected uninoculated mice, as well as mice previously inoculated with H. pertussis vaccine, from the lethal effects of subsequent injections of H. pertussis vaccine.

MATERIALS AND METHODS

H. pertussis Vaccine.—Phase I H. pertussis vaccine 80855A² (90 billion organisms per c.c.) consisted of washed bacterial cells which had been grown on sheep blood agar. It was prepared from a pool of 6 different strains of organisms and was preserved with phenol.

Mice.—White, 20 to 40 gram, male and female mice were employed. Some were obtained from the departmental mouse colony, some were purchased from dealers, but the great majority were received from Dr. J. L. Melnick of the Section of Preventive Medicine, Yale University. Mice from this latter source had previously been inoculated intracerebrally with either Lansing poliomyelitis virus and antiserum, or West Nile virus and antiserum, and had survived. Parallel experiments employing such postneutralization test (PNT), mice and normal mice revealed no significant differences in their response to...
various materials following pertussis inoculation. In most studies, two or more groups of mice were used. Randomization of the animals was accomplished by the use of a die or by a table of random numbers.

**Histamine.**—Histamine diphosphate (Eimer and Amend) was used throughout these experiments. In most cases, 1 per cent solutions were used; however, when large doses of histamine were required, 10 per cent solutions were utilized. Histamine diphosphate was dissolved in distilled water and the solution was then sterilized by passing it through an Ls Chamberland filter. Histamine was injected on the basis of milligrams of histamine diphosphate per kilogram of mouse weight. The total volume inoculated varied from 0.05 c.c., to 0.6 c.c. An experiment was performed which indicated that volume differences of this order did not influence the toxic effects of histamine.

**Sensitizing Procedure.**—A modification of the method employed by Parenfjev and Goodliné was employed. Mice were sensitized by intraperitoneal injections of H. pertussis vaccine. In order to reduce the number of toxic deaths during the sensitization procedure, one-third of the sensitizing dose was inoculated on each of three successive days. Six days after the last sensitizing injection, mice were challenged intraperitoneally with histamine, H. pertussis vaccine, or other material. Deaths occurring within a 24-hour period were noted, previous experience having indicated this to be an adequate period.

**EXPERIMENTAL**

**Inhibition of Histamine Death by Cortisone.**—Twenty to 30 gram male and female, white mice were sensitized with 0.2 c.c. of H. pertussis vaccine 80855A on each of three successive days. Six days after the last sensitizing injection, all animals were challenged with 25 mg./kg. of histamine. Eighteen hours before the challenge dose of histamine was administered the mice were divided into two groups. Test animals received 3 mg. of cortisone intramuscularly; control animals were injected with an equal volume of saline.

The data in Table I indicate that only 13 per cent of the cortisone-treated mice died from an intraperitoneal injection of histamine which was fatal to 58 per cent of the untreated control animals. The difference between the percentage of deaths in the two groups of animals is a significant one (p = < .01). It was later determined that as little as 0.6 mg. of cortisone would protect sensitized mice from an LD50 of histamine.

Since cortisone contains 1.5 per cent benzyl alcohol as preservative, and sodium carboxymethylecellulose, sodium chloride, and polyoxyethylene sorbi-

### Table I. Inhibition of Histamine Death in Sensitized Mice by Cortisone

<table>
<thead>
<tr>
<th></th>
<th>Challenge Dose of Histamine (mg./kg.)</th>
<th>Survivors</th>
<th>Deaths</th>
<th>Total</th>
<th>Per Cent of Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisone-treated mice</td>
<td>25</td>
<td>47</td>
<td>7</td>
<td>54</td>
<td>13</td>
</tr>
<tr>
<td>Control mice</td>
<td>25</td>
<td>21</td>
<td>29</td>
<td>50</td>
<td>58</td>
</tr>
</tbody>
</table>

p = < .01

*Sensitized with 0.2 c.c. of H. pertussis vaccine 80855A on each of three successive days.

†6 of 6 uninoculated mice survived this dose of histamine.
tan mono-oleate as suspending agents, these materials were tested for their protective effects against histamine. It was found that these preservatives and suspending agents possessed no inhibitory activity against the lethal effects of histamine.

Protection of Pertussis-Inoculated Mice from the Lethal Effects of H. pertussis Vaccine.—Parfentjev and his co-workers have shown that mice inoculated with H. pertussis vaccine become sensitive not only to histamine but to subsequent injections of pertussis nucleoprotein and pertussis vaccine as well. The following experiment demonstrates that cortisone protected a significant percentage of pertussis-inoculated mice from death due to subsequent injections of pertussis vaccine.

Twenty-five to 35 gram PNT mice were injected with 0.2 c.c. of H. pertussis vaccine 805855A on each of three successive days. The mice were then divided into four groups. Eighteen hours before a challenge dose of 0.1 c.c. of H. pertussis vaccine, Groups I to III received intramuscular injections of the following amounts of cortisone: Group I, 0.3 mg.; Group II, 1 mg.; Group III, 3 mg. The total volume injected was 0.2 c.c. Group IV was inoculated with this amount of saline. The challenge dose of pertussis vaccine was administered on the sixth day after the last sensitizing injection.

The data in Table II indicate that 0.1 c.c. of H. pertussis vaccine killed 74 per cent of the untreated animals, 75 per cent of the mice given 0.3 mg. of cortisone, 43 per cent of the animals injected with 1 mg. of cortisone, and only 4 per cent of the mice inoculated with 3 mg. of this adrenal steroid. Calculations based on the data in Table II showed that 1 mg. of cortisone protected a significant percentage of pertussis-inoculated mice from death due to subsequent injections of pertussis vaccine.

<table>
<thead>
<tr>
<th>AMOUNT OF CORTISONE</th>
<th>SURVIVORS</th>
<th>DEATHS</th>
<th>TOTAL</th>
<th>PER CENT OF DEATHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Mice I</td>
<td>0.3 mg.</td>
<td>5</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Test mice II†</td>
<td>1.0 mg.</td>
<td>13</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Test mice III‡</td>
<td>3.0 mg.</td>
<td>22</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>Control mice IV</td>
<td>7</td>
<td>20</td>
<td>27</td>
<td>74</td>
</tr>
</tbody>
</table>

*6 of 6 uninoculated mice survived 0.1 c.c. of pertussis vaccine.
†The difference between the percentage of deaths in Group II and the Control Group IV is a significant one (p = .01).
‡The difference between the percentage of deaths in Group III and the Control Group IV is a significant one (p = < .01).

It was of interest to determine whether cortisone would also inhibit the lethal effects of H. pertussis vaccine in uninoculated mice. If cortisone could be shown to protect uninoculated mice from toxic death due to H. pertussis vaccine, it would not be necessary to consider its prevention of death due to pertussis vaccine in mice previously injected with this vaccine on the basis of some antiallergic property it might possess.

The data listed in Table III demonstrate that 62 per cent of the untreated control animals were killed by an intraperitoneal injection of 1 c.c. of H. per-
tussis vaccine; in contrast, only 7 per cent of the cortisone-treated animals succumbed to this procedure. The difference between the two groups is a significant one (p = .01). Cortisone, therefore, protected uninoculated mice from toxic death due to H. pertussis vaccine.

TABLE III. PROTECTION OF NORMAL MICE WITH CORTISONE FROM TOXIC DEATH DUE TO H. PERTUSSIS VACCINE

<table>
<thead>
<tr>
<th></th>
<th>Survivors</th>
<th>Deaths</th>
<th>Total</th>
<th>Per Cent of Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisone-treated mice</td>
<td>15</td>
<td>1</td>
<td>16</td>
<td>7*</td>
</tr>
<tr>
<td>Control mice</td>
<td>6</td>
<td>10</td>
<td>16</td>
<td>62</td>
</tr>
</tbody>
</table>

*The difference between the percentage of deaths in the cortisone-treated mice and in the control mice is a significant one (p = .01).

The Effect of the Antihistamine Neoantergan Against Histamine and H. pertussis Vaccine.—Twenty to 35 gram PNT male and female, white mice were injected with 0.2 c.c. of H. pertussis vaccine 80855A on each of three successive days. Six days after the last sensitizing injection, the animals were divided into four groups which were inoculated in the following manner: Group I received 50 mg./kg. of the antihistamine, Neoantergan intraperitoneally, followed within 10 to 20 minutes by 25 mg./kg. of histamine; Group II was injected with distilled water (equivalent in volume to the Neoantergan solution used in Group I); followed within 10 to 20 minutes by 25 mg./kg. of histamine; Group III received 50 mg./kg. of Neoantergan followed within 10 to 20 minutes by 0.1 c.c. of H. pertussis vaccine 80855A; Group IV was inoculated with distilled water followed in 10 to 20 minutes by 0.1 c.c. of H. pertussis vaccine 80855A.

TABLE IV. NEOANTERGAN AGAINST THE LETHAL EFFECTS OF HISTAMINE AND PERTUSSIS VACCINE IN PERTUSSIS-INOCULATED MICE

<table>
<thead>
<tr>
<th>Group</th>
<th>Mice Sensitized with:</th>
<th>Injected 6 Days Later with:</th>
<th>Survivors</th>
<th>Deaths</th>
<th>Total</th>
<th>Per Cent of Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.2 c.c. of H. pertussis vaccine 80855A on each of 3 successive days</td>
<td>50 mg./kg. of Neoantergan followed by 25 mg./kg. of histamine</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>0.2 c.c. of H. pertussis vaccine 80855A on each of 3 successive days</td>
<td>Distilled water followed by 25 mg./kg. of histamine</td>
<td>13</td>
<td>17</td>
<td>30</td>
<td>56</td>
</tr>
<tr>
<td>III</td>
<td>0.2 c.c. of H. pertussis vaccine 80855A on each of 3 successive days</td>
<td>50 mg./kg. of Neoantergan followed by 0.1 c.c. of H. pertussis vaccine</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>55</td>
</tr>
<tr>
<td>IV</td>
<td>0.2 c.c. of H. pertussis vaccine 80855A on each of 3 successive days</td>
<td>Distilled water followed by 0.1 c.c. of H. pertussis vaccine</td>
<td>11</td>
<td>8</td>
<td>19</td>
<td>41</td>
</tr>
</tbody>
</table>

*6 of 6 uninoculated mice survived this dose of histamine.
†6 of 6 uninoculated mice survived this dose of H. pertussis vaccine.

The data summarized in Table IV show that 25 mg./kg. of histamine killed 56 per cent of the unprotected animals; however, no mice died from this dose
of histamine when it was preceded by 50 mg./kg. of Neoantergan. The difference between the percentage of deaths in these groups is a significant one ($p = < .01$).

One tenth of a c.c. of $H. pertussis$ vaccine was fatal to 41 per cent of unprotected mice; it was also lethal to 55 per cent of the animals given Neoantergan prior to the challenge dose of pertussis vaccine. The difference between the percentage of deaths in these latter groups is not significant.

It is apparent, therefore, that the antihistamine, Neoantergan, protected pertussis-sensitized mice from the lethal effects of histamine, but not from fatal amounts of $H. pertussis$ vaccine.

It should be noted, however, that histamine kills sensitized mice within an hour, whereas the majority of sensitized mice which succumb to injections of pertussis vaccine die in 18 to 24 hours.

**Effect of the Dosage of Neoantergan on Its Protective Action Against Histamine.**—Twenty to 35 gram PNT male and female, white mice were inoculated with 0.1 c.c. of $H. pertussis$ vaccine 80855A on each of three successive days. On the sixth day after the last inoculation, the animals were divided into four groups. Group I was injected with 1.0 mg./kg. of Neoantergan; Group II, 3 mg./kg. of this antihistamine; Group III was inoculated with 10 mg./kg. of Neoantergan; Group IV received distilled water in amounts equivalent to the volumes of antihistamine administered to Group III. In each batch of animals, these injections were followed within 10 to 20 minutes by a challenge dose of 25 mg./kg. of histamine.

**Table V. Effect of the Dosage of Neoantergan on Its Protective Action Against Histamine**

<table>
<thead>
<tr>
<th>AMOUNT OF NEOANTERGAN</th>
<th>SURVIVORS</th>
<th>DEATHS</th>
<th>TOTAL</th>
<th>PER CENT OF DEATHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test mice I</td>
<td>1 mg./kg.</td>
<td>7</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Test mice I1</td>
<td>3 mg./kg.</td>
<td>14</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Test mice III</td>
<td>10 mg./kg.</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Control mice IV</td>
<td>---</td>
<td>11</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

*The dose of histamine employed (25 mg./kg.) was not lethal to 6 of 6 uninoculated mice.

$^1$The difference between the percentage of deaths in Group II and the Control Group IV is a significant one ($p = < .01$).

$^2$The difference between the percentage of deaths in Group III and the Control Group IV is a significant one ($p = < .01$).

Calculations based on the data compiled in Table V indicate that as little as 3 mg./kg. of Neoantergan protected a significant percentage of sensitized mice from a dose of histamine which was fatal to 73 per cent of the control animals. Rose and his associates$^3$ have reported that 3 mg./kg. of Neoantergan protected 16 of 16 guinea pigs from an $LD_{100}$ of histamine (0.4 mg. of histamine base per kg.).

**DISCUSSION**

Cortisone has been shown to be effective in various allergic conditions. Clinically, the administration of this hormone has been beneficial in asthma, hay fever, urticaria, and various types of eczema. In experimental hyper-
sensitivity of animals, the use of cortisone or ACTH has produced the following results: (1) Inhibition of the tuberculin skin reaction and tuberculin shock in sensitized guinea pigs. (2) Suppression of the Shwartzman phenomenon. (3) Prevention of the development of periarteritis nodosa and cardiac inflammation in rabbits made sensitive to horse serum. (4) Inhibition of the development of the Arthus state. (5) Inhibition of anaphylaxis in the mouse.

Fischel has studied the effect of adrenal cortical hormones in relation to several steps in the development of immune or allergic reactions. He found that anaphylaxis, the local passive Arthus reaction, and other manifestations of the union of antigen with antibody were not appreciably altered. However, an inhibition of antibody production was produced by ACTH.

The reduction of circulating antibodies by ACTH and cortisone may not be the only explanation of the striking effect of these hormones in allergic conditions. Since allergic and anaphylactic reactions seem to be produced by histamine, histamine-like substances, or other metabolites, the effect of cortisone upon the lethal action of histamine in pertussis-inoculated mice was studied.

The data in Table I indicate that cortisone inhibited the fatal effects of histamine in pertussis-inoculated mice. However, cortisone is not a specific histaminic antagonist. Inflammatory reactions induced by such diverse stimuli as burns, turpentine, glycerol, tubercle bacilli, trauma, mustard oil, and formaldehyde have been attenuated or inhibited by the action of this hormone. Factors in the inflammatory response reported to be affected by ACTH and cortisone were vascular changes, leukocytic infiltration, connective tissue formation, and macrophage accumulation. Cortisone thus seems to act by altering the capacity of the host’s tissue to respond to various types of irritants. Its inhibitory action in hypersensitive states might therefore be directed against the inflammatory response induced by the type of irritants peculiar to these conditions, namely, histamine or other substances released from cells as a consequence of an antigen-antibody reaction.

In contrast to the failure of cortisone or ACTH to prevent histamine death in the guinea pig, experiments reported in this paper have indicated that cortisone could inhibit the fatal effects of histamine in mice previously inoculated with H. pertussis vaccine. This latter finding makes untenable the view that allergic reactions inhibited by cortisone do not involve histamine and that allergic reactions not affected by cortisone are due to histamine.

A possible explanation for the failure of cortisone to prevent histamine death in the guinea pig might lie in the extreme sensitivity of this animal to histamine (LD₅₀ of 0.2 to 0.4 mg./kg. of histamine base) and in the failure of various investigators to challenge the pigs with an LD₅₀ of histamine (admittedly a difficult procedure in this animal). In many cases, guinea pigs have been injected intravenously with an MLD of histamine and death occurred almost immediately. In contrast, sensitized mice were challenged intraperitoneally by the author with an LD₅₀ of histamine. Of the unprotected control
mice which succumbed to this dose of histamine, a majority expired in thirty minutes; the remainder died within 24 hours. Wilson, Booth, and DeEds\textsuperscript{17} have reported that flavonoids protected guinea pigs against an LD\textsubscript{50} of histamine but not against an LD\textsubscript{100} of this toxic amine.

It has been shown that the adrenal steroid, cortisone, and the antihistaminic, Neoantergan, protected sensitized mice from histamine death. It is quite likely that mice inoculated with \textit{H. pertussis} vaccine, and thus rendered sensitive to the lethal effects of histamine, could be used to screen other types of compounds for their activity against histamine. The guinea pig has been widely employed for this purpose. However, the use of mice previously injected with \textit{H. pertussis} vaccine would seem to be more advantageous for the following reasons:

1. Numerous experiments have indicated that it is an easy matter to determine the LD\textsubscript{50} of histamine in mice previously inoculated with \textit{H. pertussis} vaccine; mice from three different sources have been used and at least 5 different commercial Phase I \textit{H. pertussis} vaccines have been successfully employed. It is much more difficult to obtain an LD\textsubscript{50} of histamine in guinea pigs. Wilson, Booth, and DeEds\textsuperscript{17} reported that a 5 per cent change in dosage was sufficient to change an LD\textsubscript{50} to an LD\textsubscript{100} or an LD\textsubscript{1000}. They also stated that sensitivity to histamine varied from group to group of animals; in cases where the protective action of a compound was slight, negative results were obtained when the amount of histamine employed killed all or nearly all of the control animals.

2. Mice are much less expensive than guinea pigs, larger numbers of animals can be used, and results are easier to evaluate.

3. The sensitivity of normal mice to histamine is greatly increased after pertussis inoculation. The normal guinea pig, however, is naturally sensitive to histamine and there is no evidence that a guinea pig sensitized to various antigens becomes even more sensitive to this agent.\textsuperscript{18} The vital capacity of asthmatic individuals is lowered by inhalation of amounts of histamine which do not affect normal people.\textsuperscript{19} It is possible, therefore, that the increased sensitivity of mice to histamine after they have been inoculated with \textit{H. pertussis} vaccine is more analogous to human allergy than is histamine sensitivity in the guinea pig.

**SUMMARY**

Cortisone inhibited the lethal effects of both histamine and \textit{H. pertussis} vaccine in pertussis-inoculated mice. Cortisone also protected uninoculated mice from toxic death due to \textit{H. pertussis} vaccine.

The antihistamine, Neoantergan, protected pertussis-sensitized mice from the lethal effects of histamine but not from fatal amounts of \textit{H. pertussis} vaccine.

The advantages of using pertussis-inoculated, histamine-sensitive mice rather than guinea pigs in the screening of compounds which might be active against histamine are discussed.
REFERENCES