ON A POSSIBLE NEW KIND OF TOXIC SUBSTANCE PRODUCED BY BORDETELLA PERTUSSIS

The purpose of this preliminary report is to present some properties of a certain kind of toxic substance produced by Bordetella pertussis.

In the course of a study on the toxicity test of pertussis vaccine, the authors had a chance to compare the pertussis reference vaccine, BD 10257*, prepared for a comparative toxicity test by a committee from the Biological Section of the Pharmaceutical Manufacturers Association, U. S. A. (Piersma et al., 1962) with our reference vaccine for use in toxicity test. And the vaccine BD 10257 was found to be different from our vaccine in toxic responses in mice.

As shown in Fig. 1, the body weight of mice inoculated with the Japanese reference vaccine, O-VII (Kurokawa et al., 1962), decreased first and exceeded the body weight preceding inoculation regardless of the doses inoculated. On the other hand, with BD 10257 the decrease of the body weight of mice occurred not only within one day but also in the later period again.

In addition to the difference in the body weight change there was also a difference in death time distribution, which probably corresponds to the patterns of body weight change. The deaths of mice inoculated with larger doses of the vaccine O-VII occurred only within two days after the inoculation, while deaths of mice given corresponding doses of BD 10257 reappeared a few days later (Fig. 2).

These patterns of responses observed with BD 10257 have never been experienced so far with pertussis vaccines produced in Japan, and suggested that the vaccine BD 10257 contained a certain kind of toxic substance responsible for the late appearing toxicity (hereafter abbreviated as LAT) besides that for the early appearing toxicity (EAT), which seemed to be contained in both vaccines tested. Another evidence in favor of this view was given by the next experiment. The vaccine BD 10257 was separated into precipitate and supernatant by centrifugation, and the supernatant alone showed a similar pattern of the body weight change to that of the whole vaccine (Fig. 3). These data suggested that the LAT was contained in the supernatant rather than in the cell component of the vaccine.

In addition, it was found that the strain No. 3779B-L2S5** which was employed for preparing the vaccine BD 10257, produced the suggested toxic factor responsible for LAT. Then further detailed studies on the toxic factor was begun using this culture.

A 24 hr culture grown in the Cohen-Wheeler's medium (Cohen, and Wheeler, 1946) containing 0.02 % carbon powder on a shaker was sterilized with 0.01 % merthiolate for five to seven days at room temperature, and then the cells were removed by centrifugation at 10,000 rpm for 20 min. Practically all of the toxic factor has been

* For supplying the vaccine the authors are grateful to Dr. H. D. Piersma, Lederle Laboratories, and Dr. J. Kaneko, Takeda Pharmaceutical Ind. Ltd.

** The authors are indebted to Dr. H. D. Piersma and Dr. A. H. Dettwiler, Eli Lilly and Company, for supply of this strain.
Mice inoculated intraperitoneally with 0.5 ml of each vaccine doses were weighed every day, and differences (ordinate) from starting body weight were taken. Conventional ddS mice (body weight 14-16 gr) were used, as well as other experiments in the report. Each point in Figure represents an average of 10 female mice.

The toxic factor was concentrated in the insoluble fraction Pr 1 as shown in Fig. 4. Little loss in body weight of mice inoculated with Pr 1 was seen within 2 days but a marked body weight loss was observed thereafter, while with the saline soluble fraction derived from the ammonium sulphate precipitation the body weight loss appeared only within one day.

As the late appearing toxic responses in mice may be due to an infection, bacteriological examination of heart blood and spleen from moribund mice inoculated with the fraction Pr 1 was made at times on the blood agar. In no case could any mouse with septicemia be found.

The toxic complexity of _Bordetella pertussis_ is well known. Of the various kinds of biologically active substances that have been reported, the most interesting ones are the protective antigen(s), histamin sensitizing factor, heat labile toxin and heat stable toxin, or endotoxin. Relationships among these substances and the suggested toxic
factor responsible for LAT are of particular interest. In preliminary, from gross chemical composition and immunological and biological properties of further purified preparations from the supernatant of the culture of BD 10257, the suggested toxic factor does not seem to be identical with the endotoxin nor with the heat labile toxin. Though detailed data will be presented in separate papers, only one evidence is given here because it is of particular importance whether the suggested toxic factor is identical with the endotoxin or not. Although rabbits are highly susceptible to the lethal effect of Pr 1, hyperimmunized sera could be obtained by subcutaneous administrations of gradually increasing amounts of the fraction over a long period, starting with 0.005 ml of the fraction. The body weight loss of mice with Pr 1 was neutralized by sera thus obtained, while sera from rabbits hyperimmunized with thoroughly washed cells of No. 3779 B–L2S5 could not neutralize the toxic effect of Pr 1.

From these data it is likely that there exists a new sort of toxic substance produced by at least a certain strain of *Bordetella pertussis*.

Another particular interest brought about by the data presented here is concerned with the testing method for freedom from toxicity of pertussis vaccine. Though the

### Fig. 2. Death time distribution of mice after inoculation of vaccine.

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>Dose</th>
<th></th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

0.5 ml of each vaccine was intraperitoneally inoculated.

- • Dead
- O Survival
Fig. 3. Body weight changes of mice inoculated with the whole vaccine, the supernatant and the reconstituted precipitate derived from the BD 10257 vaccine.

BD 10257 vaccine was centrifuged at 10,000 rpm for 20 minutes. The supernatant was removed and the precipitate was resuspended in saline of the original volume. Twelve mice for each sample were used.


Fig. 4. The body weight changes of mice inoculated with fraction obtained by ammonium sulfate fractionation from cultural supernatant.

Solid line: Culture supernatant. Dotted line: Pr 1 fraction diluted 30 folds. Brocken line: Saline soluble fraction diluted to a volume of original culture supernatant.

Each point in Figure represents an average of 12 mice.
establishment of relationships between each of the various kinds of toxicities of pertussis vaccine determined by laboratory methods and each of various kinds of untoward reactions observed in human beings is, of course, the final requisite for the purpose, one of possible approaches is to make clear the entity responsible for each of known toxicities observed in experimental animals. The toxicity appeared in the early period is considered to be mainly due to the endotoxin of *Bordetella pertussis*. Lipopolysaccharide extracted from the organisms offered an exactly similar effect to vaccine (Iwasa et al., to be published). The toxicity of the pertussis vaccines produced in Japan seems to be attributable mainly to this type of toxin. On the other hand, according to Pittman and Cox (1965), with a few pertussis vaccine preparations tested by them weight gain is slow or nil and late deaths occur, on which the U. S. A. mouse toxicity test amended in 1961 based. And they declare that this “type of reaction is different from that induced by other gram-negative bacterial vaccines when compared on an equivalent endotoxin doses”.

Our suggested toxin for LAT might provide with a substantial base to this view, though additional evidences will have to be accumulated.

This work is supported in part by a grant from the Research Committee on Combined Vaccine (Chairman: Dr. S. Someya).

REFERENCES


Department of General Biologics Control, National Institute of Health, Tokyo  
MASAMI KUROKAWA  
SABURO IWASA  
SETSUJI ISHIDA

Received: May 25th, 1965