NOTE

STANDARDIZATION AND CONTROL OF TOXOID COMPONENTS IN THE COMBINED VACCINE (DPT)

The previous paper (Yamamoto et al., 1972) stated that the potency of tetanus toxoid in the combined vaccine (DPT) varied greatly when assayed against the current National Standard Tetanus Toxoid (plain, NSTT) and that the latter might not be suitable as the reference for the assay of the toxoid component in combined vaccines. Since the fact seemed to raise a serious problem about the validity of the current assay method (called MR64 method), another experiment was conducted to confirm the previous finding by using NSTT and two combined vaccines, DPT 368 and DPT 168/268SP. The first two were used in the previous experiment. The last one was a freeze-dried combined vaccine deprived of pertussis cells by centrifugation and added with normal guinea pig serum at 20% to facilitate freeze-drying. On reconstitution with saline, the sample contained 50 Lf and 9 Lf of diphtheria and tetanus toxoids, respectively. The results are shown in Table I. Summarized data of the former experiments are shown in Table II for comparison. These results show clearly the inadequacy of the MR64 method for the potency test of tetanus toxoid in combined vaccine. The fact may explain why discrepancies were often observed in duplicate tests by National Institute of Health (NIH) and manufacturers in Japan (Yamamoto et al., 1972).

Such discrepancies may not be rare in the bioassay of biological products (Hardegree, Pittman and Maloney, 1972; Mussett and Sheffield, 1973). On the other hand, the need for the accurate potency test of the biological products important for public health increased recently to avoid the overuse of the vaccine. Therefore, improvement of the assay method to minimize such discrepancies is urgently required for production and control of the combined vaccine DPT. A clue to improve the method is suggested in Tables I and II. It is evident that the relative potencies to a combined vaccine were less variable than those determined against NSTT (plain). Therefore, DPT 368 was tentatively selected as the reference for potency tests of the toxoid components of other combined vaccines, since it had been tested repeatedly in our laboratory and used in the field trial carried out by the Research Committee on Pertussis and Combined Vaccine (Someya et al., 1972). However, difficulty was encountered to assign unitage of the tetanus component of the vaccine, when we tried to correlate it somehow to the current standard preparation (plain), as stated above. We finally decided to assign 200 protective units (PU) to the tetanus component of DPT 368, the value calculated relatively to the combined vaccine (called DPT RI68 in Table II) which had been used in the extensive collaborative study and had a potency of 200 IU/ml (van Ramshorst, Sundaresan and Outschoorn, 1972). This seemed reasonable since the variation in the relative potency of DPT 368 to that of DPT RI68 was not significant (Table II). On the other hand, a value of 43.2 IU/ml was obtained
# TABLE I

**Potencies of tetanus toxoid in combined vaccine DPT**

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Potency in international units</th>
<th>Potency of 168/268SP to DPT 368</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPT 368</td>
<td>DPT 168/268SP</td>
</tr>
<tr>
<td>I</td>
<td>Invalid due to inadequate animal response</td>
<td>N.D.</td>
</tr>
<tr>
<td>II-1</td>
<td>112 (63-200)</td>
<td>N.D.</td>
</tr>
<tr>
<td>II-2</td>
<td>N.D.</td>
<td>15 (11-27)</td>
</tr>
<tr>
<td>III</td>
<td>185 (116-295)</td>
<td>25 (16-40)</td>
</tr>
<tr>
<td>IV</td>
<td>53 (28-100)</td>
<td>17 (9.6-30)</td>
</tr>
<tr>
<td>V</td>
<td>76 (38-152)</td>
<td>17 (8.7-31)</td>
</tr>
<tr>
<td>VI</td>
<td>82 (54-124)</td>
<td>27 (18-41)</td>
</tr>
<tr>
<td>VII</td>
<td>27 (16-44)</td>
<td>12 (7.1-19)</td>
</tr>
<tr>
<td>VIII</td>
<td>54 (32-90)</td>
<td>17 (9.8-31)</td>
</tr>
<tr>
<td>Weighted mean</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

1) Three graded doses of each preparation were inoculated to groups of 10 guinea pigs. NSTT (plain) was included in all experiments. Samples were diluted with phosphate (M/60) buffered saline containing 0.02% gelatin (GPB). Animals were challenged with 50 LD50 of a tetanus toxin 4 weeks after immunization. The results were calculated by the score method reported previously (Yamamoto et al., 1972). Figures in parentheses show the fiducial limits at p = 0.95.

N.D.: Not determined.

2) Determined relatively to the potency of NSTT (plain).

3) Calculated by taking the potency of DPT 368 as 100.

4) Calculated by the method described by Finney (1952).

# TABLE II

**Variation in the relative potency of tetanus toxoid in combined vaccine DPT**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Diluent</th>
<th>Potency in international units</th>
<th>Potency of DPT 368 to DPT R168</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DPT 368</td>
<td>DPT R168</td>
</tr>
<tr>
<td>1</td>
<td>Saline</td>
<td>24-194 (10)</td>
<td>39-167 (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$X^2 = 49.05$</td>
<td>$X^2 = 33.0$</td>
</tr>
<tr>
<td>2</td>
<td>GPB1)</td>
<td>57-214 (10)</td>
<td>21-238 (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$X^2 = 55.74$</td>
<td>$X^2 = 65.6$</td>
</tr>
<tr>
<td>Weighted mean and fiducial limits at p = 0.95</td>
<td>N.D.</td>
<td>N.D.</td>
<td>102, 96.7-106.5</td>
</tr>
</tbody>
</table>

1) Taken from the previous experiment (Yamamoto et al., 1972). Only ranges of the potencies are shown in the Table. Figures in parentheses show the number of the experiments.

N.D.: Not determined.

2) Refer to the footnote 2) to Table I.

3) Calculated by taking the potency of DPT R168 as 100.
as the mean potency of the tetanus component of DPT 368 from five assays carried out in NIH by the MR64 method with NSTT (plain) as the reference and GPB as the diluent. The difference between the two values, 0.6653 (=log 200–log 43.2), may be useful to estimate the potency in new PU of other combined vaccines which were assayed in NIH by the MR64 method. The logarithm of the estimated new potency (in PU) can be obtained by adding 0.6653 to the logarithm of the potency obtained by the MR64 method. This procedure was applied to several combined vaccines which had been used in the field trial (Murata, Someya and Kaneko, 1970). Figure 1 shows the circulating tetanus antitoxin among children having received three injections of combined vaccines DPT containing different amounts of tetanus toxoid but almost the same amount of diphtheria toxoid. The total amounts of tetanus toxoid of three injections are shown in the figure as the injection doses in volume were not always the same. The results show clearly that a straight line was obtained between the amount of vaccine in PU and the circulating antitoxin of children. A combined vaccine containing 100 PU per total human doses may give sufficient immunity to tetanus (about 2.0 IU/ml of antitoxin) in children. Therefore, a value of 60 PU/ml may be used to control the potency of the tetanus component of the combined vaccine*.

As regards the potency of the diphtheria component of DPT 368, the unitage relative to DPT RI68 could not be calculated since no test was carried out in the collaborative study (van Ramshorst et al., 1972). We, therefore, tentatively decided to use the mean value (35 IU/ml) determined relatively to the current National Standard Diphtheria Toxoid (plain, NSDT) as shown in Table III. This seemed practically useful since the variation in the potencies against NSDT was less significant even

Fig. 1. Relationship between the circulating tetanus antitoxin in children and the amount of tetanus toxoid in combined vaccine (DPT).

Children of 3-6 months of age were inoculated in three injections with the combined vaccine DPT at intervals of a month and bled a month after the last injection. Tetanus antitoxin was determined by the mouse method at the L+ /1,000 level.

* Three doses of 0.5 ml shall be used for children.
if determined under different experimental conditions (Table III) than in those of the
tetanus component and since most of the combined vaccines (158 out of 175 samples)
recently produced in this country had potencies of about 15-60 IU/ml of the diph-
theria component. Therefore, 35 PU/ml was assigned as the new protective units to
the diphtheria component of DPT 368.

TABLE III
Potency tests of diphtheria toxoid in combined vaccine DPT

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Diluent</th>
<th>Potency of DPT 368 in international units and fiducial limits at 0.95</th>
<th>Potency of 168/268SP to DPT 368</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>GPB</td>
<td>29, 11-76</td>
<td>131, 54-321</td>
</tr>
<tr>
<td>VII-1</td>
<td>GPB</td>
<td>27, 15-48</td>
<td>-</td>
</tr>
<tr>
<td>-2</td>
<td>Saline</td>
<td>30, 16-57</td>
<td>-</td>
</tr>
<tr>
<td>-4</td>
<td>GPB</td>
<td>35, 20-60</td>
<td>-</td>
</tr>
<tr>
<td>VIII</td>
<td>GPB</td>
<td>68, 34-130</td>
<td>73, 44-122</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( \chi^2 = 4.98 )</td>
<td>( \chi^2 = 5.54 )</td>
</tr>
<tr>
<td>Weighted mean</td>
<td></td>
<td>35.4, 27.1-46.6</td>
<td>65.8, 45.3-95.5</td>
</tr>
</tbody>
</table>

1) Three graded doses of each preparation were inoculated to groups of 10 guinea pigs. NSDT
(plain) was included in all experiments. Animals were bled 4 weeks after immunization.
Antitoxin of the sera were titrated by the rabbit ic method at the LR/2,000 level.
2) Determined relatively to the potency of NSDT (plain).
3) Refer to the footnote 1) to Table I.
4) Calculated by taking the potency of DPT 368 as 100.

Children having received three injections (0.5, 1 and 1 ml) of the combined vac-
cine containing more than 20 IU/ml (by MR64 method) of diphtheria toxoid showed
circulating diphtheria antitoxin of approximately 1-2 IU/ml (unpublished data), a value
being sufficient to protect against diphtheria. Since DPT 368 is kept in a fluid state,
we are going to replace it by another stable (freeze-dried) preparation which shall be
standardized against the former. The new proposed reference preparation for the
combined vaccine shall be so prepared as to contain 20 PU/ml and 60 PU/ml with
respect to the diphtheria and tetanus components, respectively.

REFERENCES
London.

HARDEGREE, M. C., PITTMAN, M. AND MALONEY, C. J. (1972): Influence of mouse strain

MUSSETT, M. V. AND SHEFFIELD, F. (1973): A collaborative investigation of methods pro-
posed for the potency assay of adsorbed diphtheria and tetanus toxoids in the European

MURATA, R., SOMEYA, S. AND KANEKO, J. (1970): Standardization of tetanus toxoid com-


ADDENDUM

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