Antibody Response to Successive Booster Doses of Tetanus Toxoid in Adults

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A study of the rise and fall in circulating tetanus antitoxin in a group of 15 adults after basic and booster immunization indicates that although individuals vary greatly in their responsiveness to toxoid, the falloff in antitoxin appears to be fractionally constant for each individual, and over periods of 5 to 6 years appears to be the same after successive doses. The low level of antitoxin noted in some individuals after basic immunization with a plain toxoid preparation cannot always be rectified by the use of an adsorbed toxoid as a booster. Results indicate that routine boosters need not be administered more frequently than every 10 to 20 years, provided an adsorbed toxoid has been used to initiate active immunization.

Although optimal time intervals have been established for the basic course of tetanus toxoid (3), the frequency with which subsequent booster doses should be given remains controversial (14, 30). Some have even questioned whether regular boosting is necessary for all persons (16, 21). Published data on the persistence of tetanus antitoxin in the circulation of the actively immunized show many inconsistencies, and these have undoubtedly been a major cause of the present confusion and lack of agreement. Such inconsistencies have been attributed to dissimilarity between the groups of individuals studied and to use of single rather than paired assays to estimate falloff in immunity (25). To obtain more precise information of the persistence of active tetanus immunity, the rise and fall of antitoxin in the same individual has been studied after successive booster doses of tetanus toxoid.

MATERIALS AND METHODS

During 1958 to 1959, a campaign to actively immunize employees against tetanus was undertaken at the Commonwealth Serum Laboratories, Melbourne. Of 71 employees given a basic course of three doses of tetanus toxoid (either aluminum phosphate adsorbed containing 8 Lf (Limes flocculation) per 0.5-ml dose or plain formol containing 16 Lf per 1-ml dose), 41 received a booster dose in 1964. Fifteen of the group, comprising eight females and seven males, were still available in 1970 for further assessment of their immunity. Ages ranged from 17 to 49 years at the beginning of immunization. The intervals between the doses of the basic course were 6 weeks and 6 months, respectively. None had knowingly received tetanus toxoid previously.

For basic immunization, four subjects received the adsorbed preparation (A), and the remainder received the plain formol preparation (P). For the first booster, ten subjects received the adsorbed preparation, and five received the plain formol preparation.

Venous blood samples were taken 2 to 3 weeks and 5 years after the completion of the basic immunization course, and again 3 weeks and 6 years after the booster dose. The survey thus covered a period of approximately 12 years. Four of the subjects received a second booster of adsorbed toxoid, and a further sample of blood was taken 3 weeks later from each.

The separated serum was titrated for tetanus antitoxin according to the method of Glenny and Stevens (8), with single or paired mice as the test animal on each dilution. The lowest unitage tested for was 0.01 IU/ml. An upper limit of 10 to 15 IU/ml was set for the first (immediate post-basic immunization) sample, but thereafter no upper limit of titration was set, the range being extended whenever an end point could not be reached with a series of dilutions. Unique values were calculated according to the day of death of the mice.

RESULTS

Individual assay results are given in Table 1. After basic immunization, the geometric mean tetanus antitoxin titer was calculated to be 2.1 IU/ml of serum, whereas after booster immunization the mean titer was 10.0 IU/ml. The post-toxoid titers of the four subjects who received a second booster are shown in the last column in Table 1.

Individual antibody responses are indicated in Fig. 1. Antitoxin was not measured in any of the subjects before the conclusion of the basic immunization course.
TABLE 1. Tetanus antitoxin titers in 15 subjects after basic and booster immunizations

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age* (years)</th>
<th>Immunization</th>
<th>Titer at 2-3 weeks</th>
<th>Titer at 5 years</th>
<th>Titer at 3 weeks</th>
<th>Titer at 6 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>46</td>
<td>A</td>
<td>1.0</td>
<td>0.1</td>
<td>A</td>
<td>16.0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>31</td>
<td>A</td>
<td>&gt;12.5</td>
<td>0.8</td>
<td>A</td>
<td>35.0</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>49</td>
<td>A</td>
<td>&gt;10.0</td>
<td>1.35</td>
<td>P</td>
<td>16.0</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>30</td>
<td>A</td>
<td>&gt;10.0</td>
<td>0.6</td>
<td>P</td>
<td>520.0</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>31</td>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>A</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>41</td>
<td>P</td>
<td>0.4</td>
<td>&lt;0.01</td>
<td>A</td>
<td>0.4</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>41</td>
<td>P</td>
<td>0.4</td>
<td>&lt;0.01</td>
<td>A</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>27</td>
<td>P</td>
<td>0.4</td>
<td>0.06</td>
<td>A</td>
<td>1.6</td>
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<td>9</td>
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<td>24</td>
<td>P</td>
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<td>0.03</td>
<td>A</td>
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<tr>
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<td>47</td>
<td>P</td>
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<td>A</td>
<td>12.0</td>
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<tr>
<td>11</td>
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<td>26</td>
<td>P</td>
<td>2.5</td>
<td>0.08</td>
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<td>18.0</td>
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<tr>
<td>12</td>
<td>F</td>
<td>29</td>
<td>P</td>
<td>&gt;15.0</td>
<td>12.0</td>
<td>A</td>
<td>28.0</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>34</td>
<td>P</td>
<td>&gt;15.0</td>
<td>0.19</td>
<td>P</td>
<td>36.0</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>29</td>
<td>P</td>
<td>10.0</td>
<td>0.05</td>
<td>P</td>
<td>55.0</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>42</td>
<td>P</td>
<td>0.4</td>
<td>0.04</td>
<td>P</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Geometric mean titer*: 2.1 0.12 10.0 0.3
Median titer: 2.0 0.1 16.0 0.6

* At start of basic immunization course.
* A, adsorbed preparation; P, plain formol preparation.
* For calculation of the geometric mean, titers preceded by < or > sign are assumed to be one-third or three times the value, respectively.

FIG. 1. Increase in antitoxin after basic (first column), first booster (second column), and, in the case of four subjects, second booster (third column) immunizations in 15 subjects given adsorbed (A) or plain (P) toxoid at intervals of 5 and 6 years. The lower and upper ends of each column represent the pre- and postinoculation titers, respectively. Dotted lines indicate that an absolute end point was not reached for the particular assay.

Individual falloff slopes for the seven subjects for whom an absolute antitoxin value was available for each titration after both basic and booster immunizations were constructed by joining the two titration points recorded after the two phases of immunization (Fig. 2). Because of the apparent similarity of the individual slopes, it seemed legitimate to determine an
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The average value for the slopes based on the geometric mean titer of all the observations for which there were unique end points. Values for each individual slope have been calculated according to the formula \( b = (\log t_0 - \log t_Y)/Y \), where \( b \) is the value of the slope, \( t_0 \) is the titer at 2 to 3 weeks, \( t_Y \) is the titer at time \( Y \) (years), and \( Y \) is number of years. The mean value of the seven slopes after basic immunization was 0.248, whereas the mean of the 14 slopes after booster immunization was 0.247. The standard deviation of the 21 individual slopes was ±0.07. It was not possible to determine a slope for subject no. 5 after either basic or booster immunization, since the antitoxin titer was below the minimal level of assay at the end of each falloff period. Falloff in antitoxin is shown graphically in Fig. 3.

**DISCUSSION**

Although the numbers are small and the statistical analysis is limited to some extent because an end point was not reached in all of the titrations, thus eliminating the worst and some of the best responders from the estimates of slopes, nevertheless certain conclusions can be drawn. For instance, because of the similarity of the individual slopes after the two phases of immunization (Fig. 2), it could be concluded that antitoxin falloff is fractionally constant for each person. Furthermore, the closeness of the values for the mean slopes, represented graphically in Fig. 3, indicates that falloff occurs at a similar rate after successive recall doses of toxoid.

Only one other study has been found where serum titrations have been made on the same individual after both basic and booster tetanus immunization (26). However, it is difficult to make comparisons because so many of the assay results in this earlier paper were expressed as a range of titration varying from two- to 1,000-fold, whereas the period of falloff covered no

![Fig. 2. Individual falloff slopes for subjects 1, 8-11, 14, and 15.](image)

![Fig. 3. Falloff in antitoxin after basic and booster immunization based on geometric mean titer.](image)
more than 15 months and, moreover, was of variable duration after the basic course.

Reliable information of tetanus antitoxin fall-off after either basic or booster immunization in the same individual is limited to a few reports (6, 12, 23, 24, 29). However, apart from one study (24) in which paired titrations were made in a group of children at intervals of 6 to 8 and 12 to 14 years after basic immunization, none of these reports spans a period of more than 5 years. Other estimations of long-term decline in tetanus antitoxin in humans have been based on the results of single assays, and these have been made on dissimilar groups at different times after basic or booster immunization using various types of toxoid and dose schedules (1, 2, 5, 7, 9–11, 13–15, 17–20, 22, 27, 28, 30). Not unexpectedly, analysis of the single assay results show considerable variability, not only in the individual and mean titers, but also in the proportion with nonprotective immunity at comparable times after immunization.

It is now generally agreed that a tetanus antitoxin titer of 0.01 IU/ml of serum affords adequate protection against tetanus (31). One subject failed to acquire a protective level of immunity after the third dose of the basic course of plain toxoid. This finding suggests either a poor antigen or a poor responder, but since this subject was healthy, had a normal complement of immunoglobulins, and responded normally to other antigens, it is more likely that the antigenicity of the toxoid was at fault. The fact that five other individuals also failed to acquire a satisfactory level of immunity (>0.1 IU/ml) after basic immunization with the plain toxoid preparation supports the hypothesis that this particular toxoid was of inferior quality. The quality of the antigen used to initiate active immunization has been shown to greatly influence subsequent antibody responses to the antigen (4). Comparative studies (28, 30) have clearly demonstrated that adsorbed tetanus toxoids are antigenically superior to plain tetanus toxoids. In this study, all four subjects who received the adsorbed preparation for basic immunization had serum antitoxin titers of 1 IU/ml or greater after the course, and all subsequently responded well to the booster dose of either plain or adsorbed toxoid. In three of the six who responded weakly to the basic immunization course of plain toxoid (i.e., post-titer of <0.1 IU/ml), neither of two subsequent booster doses of adsorbed toxoid caused any significant rise in the level of antitoxic immunity.

Prolongation of the mean slopes in Fig. 3 shows intersection with the minimal protective level at 9.3 years after basic immunization and at 12 years after boosting. However, actual protection is likely to be of even longer duration, since a straight line oversimplifies the true falloff curve and is inclined to underestimate the duration of protective immunity (18).

Frequency of routine boosting will depend on two factors: (i) the duration of protective immunity and (ii) the capacity to respond to a recall dose. Since approximately half the cases of tetanus develop from wounds which are considered so trivial that medical attention is not sought, the importance of maintaining a protective level of active immunity at all times is obvious. Although tetanus toxoid is recognized to be one of the safest immunizing agents, there is growing concern that excessive use may cause undesirable hypersensitivity reactions (5).

The capacity of the actively immunized to respond to a recall dose of tetanus toxoid is retained for at least 20 years (28). Although anamnesis has been found to last longer than 20 years in some individuals (5, 7, 20), apparent failures (18, 30) after this lapse of time indicate that it may not be lifelong in every case.

These results support the growing evidence that, provided the basic immunization schedule is carried out with an effective toxoid at properly spaced intervals (3), the first routine booster dose of toxoid need not be given within 10 years of the completion of the basic course (1). Since subsequent boosting is likely to raise the antitoxin titer and thus prolong the duration of protective immunity (18), and since the actively immunized individual retains the capacity to respond to tetanus toxoid for at least 20 years, an interval of 15 to 20 years could safely elapse before a second or third routine booster need be given, provided an effective toxoid has been used. These recommendations are, of course, not applicable to emergency wound boosting. They are intended for the healthy community at large and cannot be expected to cover the rare individual with a defective immunological system or the "poor" responder who has not had the benefit of an efficient toxoid for basic immunization.

ACKNOWLEDGMENTS
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LITERATURE CITED
Grune and Stratton, New York.