LABORATORY DATA SUPPORTING THE CLINICAL TRIAL OF ANTI-RABIES SERUM IN PERSONS BITTEN BY A RABID WOLF

KARL HABEL, M.D.
Chief, Basic Studies Section, Laboratory of Infectious Diseases, National Microbiological Institute, U.S. Department of Health, Education and Welfare, Bethesda, Md, USA
Member, Expert Panel on Rabies, World Health Organization

HILARY KOPROWSKI, M.D.
Assistant Director, Viral and Rickettsial Research Section, American Cyanamid Company Research Division, PEARL RIVER, N.Y., USA
Member, Expert Panel on Rabies, World Health Organization

Manuscript received in September 1955

SYNOPSIS

Five individuals severely exposed to rabies by wolf bite and treated with a course of phenolized vaccine alone showed no demonstrable antibodies in their sera until the nineteenth day following the start of treatment. Three of these five individuals died of rabies.

On the other hand, twelve individuals similarly exposed, who received antirabies serum plus a course of phenolized vaccine, had demonstrable antibodies early in and throughout the period of observation. One individual who received one dose of serum plus a course of vaccine died of rabies.

In view of these results, it is apparent that antibody demonstrable early in and throughout the treatment period, and obtained by the combined use of serum and vaccine, is more effective in preventing rabies after severe exposure than is a course of vaccine alone.

Certain laboratory tests were carried out in order to provide supporting quantitative data in the clinical trial of the effectiveness of antirabies serum combined with a course of antirabies vaccine reported in the preceding paper (see page 747). Determination of the potency of the serum and vaccine used in the trial was necessary in order to evaluate the clinical results properly. Titres of neutralizing antibody in the sera of patients bled serially at intervals after the start of treatment were determined in an attempt to correlate the outcome in individual cases with the level of antibody present.
in their sera. As pointed out in the preceding paper, only those cases suffering from head and neck wounds (series A, B, and C, and case No. 27) were subjected to intensive laboratory investigation.

**Material and Methods**

*Virus strain*

For the neutralization test the standard fixed challenge virus strain (CVS)* was used in all experiments. The mouse-brain suspension infected with the CVS was stored in the frozen state until ready to be used.

*Neutralization test*

Undiluted and serial five-fold dilutions of test sera were mixed in equal volumes with a dilution of mouse-brain suspension (final serum dilutions, 1:2, 1:10, etc.) containing 3.46 LD₅₀ of CVS. The serum-virus mixture were incubated for 90 minutes at 37°C before intracerebral injection into Swiss albino mice. Five mice were used for each serum dilution. The test was recorded fourteen days after inoculation, and the 50% end-point of the neutralizing serum was calculated by the method of Reed & Muench.

*"Standard" serum containing a known level of antitoxin antibodies was included in every test.

All serum samples were submitted to parallel tests in two different laboratories, and the results were found to be comparable. A detailed description of the technique of the neutralization test employed can be found on page 54 of the manual, Laboratory Techniques in Rabies.5

*Vaccine potency test*

Samples of vaccine used in the treatment of patients were submitted to a standard type of label potency test.*

**Results**

*Potency of serum*

When diluted for neutralizing antibody level in comparison with the proposed International Standard for Antibodies Serum,* the titre of serum 7.995-7A, used in the control test was 1:1000 and that of the standard was 1:352. These results are shown in Table I.

*Results given as the number of mice dying over the number of mice inoculated.

**Potency of vaccine**

The placebo and sheep-brain vaccine used in this clinical trial is described in the preceding paper (see page 767). A label potency test performed on the vaccine at the Institute Pasteur at Tehran showed a protective power of 2.8 mg (protection against 621 LD₅₀) of virus. The vaccine was tested for potency approximately two months after its expiration date, and the authors point out that the vaccine was tested at the time of its use it would probably have shown a result comparable to that of other lots of vaccine prepared during the same period—namely, 3.3 mg (see page 769).

**Antibody levels in patients**

The specific titre of neutralizing antibody in the samples of blood drawn at various intervals in the course of treatment of the 17 persons bitten in the head or neck are shown in Tables II, III, and IV for Series A, B, and C respectively.

*Series A.* Two doses of serum were administered together with a course of vaccine, and all five individuals had a definite level of antibody within the first 5 days as a result of passive immunity from the serum injections. These intermediate antibody levels were maintained through the 21st day in all individuals, one of them (A3) rising to a high level. Subsequently, antibody appeared to drop to lower levels by the 53rd day in all cases except A5, who maintained a high level even at this late date. All these patients survived.

*Patient 27,* whose antibody levels are also given in Table II, was, as indicated in the preceding paper (see page 752), a case of especially severe exposure and for this reason received six doses of serum during the first 12 days, together with a course of vaccine. It can be seen that this patient had a high level of antibody throughout the period of observation. He also survived.

---

*Note: The text includes references and footnotes which are not transcribed here.*
### Table II. Neutralizing Antibody Levels in Sera of Patwion Exposed to Head and Neck Bites of Rabid Wolf. Series A, Treated with Two Doses of Serum and Complete Course of Vaccine

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Day</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>10</th>
<th>14</th>
<th>18</th>
<th>22</th>
<th>26</th>
<th>30</th>
<th>34</th>
<th>38</th>
<th>42</th>
<th>46</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>A3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>A4</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>A5</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>A6</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>A7</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
</tbody>
</table>

### Table III. Neutralizing Antibody Levels in Sera of Patients Exposed to Head and Neck Bites of Rabid Wolf. Series B, Treated with One Dose of Serum and Complete Course of Vaccine

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Day</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>10</th>
<th>14</th>
<th>18</th>
<th>22</th>
<th>26</th>
<th>30</th>
<th>34</th>
<th>38</th>
<th>42</th>
<th>46</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>B3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>B4</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>B5</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>B6</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>B7</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
</tbody>
</table>

### Table IV. Neutralizing Antibody Levels in Sera of Patients Exposed to Head and Neck Bites of Rabid Wolf. Series C, Treated with Complete Course of Vaccine Only

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Day</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>10</th>
<th>14</th>
<th>18</th>
<th>22</th>
<th>26</th>
<th>30</th>
<th>34</th>
<th>38</th>
<th>42</th>
<th>46</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>C2</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>C3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>C4</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>C5</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>C6</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>C7</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>16</td>
<td>22</td>
<td>28</td>
<td>34</td>
<td>40</td>
<td>46</td>
<td>52</td>
<td>58</td>
<td>64</td>
</tr>
</tbody>
</table>

**Series B:** One dose of serum was given at a course of vaccine. It can be seen from Table III that all patients had definite levels of antibody during the first 5 days as a result of the serum injection. However, by the 21st day, four of the six individuals had low antibody titers, which then continued low. One patient (B1), the only death in this series, failed to develop more than a trace of antibody beyond the seventh day. Only B5 responded with a high antibody level in the later blood samples.

**Series C:** The five patients in this series received a course of vaccine but no serum, and none showed antibodies before the 19th day. However, three developed definite titers between the 21st and 25th days. Two of these three patients died. Two other patients failed to develop any demonstrable antibody during the period of observation. One of these died of rabies; the other survived. The two highest levels of antibody in this group were obtained from fatal cases shortly before death.

### Discussion

The important point in evaluating the results of these antibody studies is the relationship of the antibody levels in an individual's serum to the subsequent clinical outcome. It must be realized that at least three different factors may affect the immunological response of an individual who has been exposed to rabies virus and is receiving treatment.

First, the passively administered specific antibody from the serum injection produces a low or intermediate level of antibody early (depending on the potency of the serum), and this level tends to decrease gradually as a result of absorption and destruction. It must be remembered that serum from a heterologous species (in this case rabbits) also acts as an antigen, and, as the humoral responses with the formation of antibodies to this heterologous protein, the virus with its rabbit antibody is gradually destroyed. At the same time, passive antibody is present early in the course of treatment, and antibody to the form of vaccine is also present, and possibly antibody from multiplying virus introduced at the time of the bite. Parallel studies in normal persons (shortly to be published in this journal) have indicated that there is no serious interference between this antibody and antigen derived from the vaccine, whereas for serum to be clinically effective antibody must inhibit the multiplication of the street virus, when it is present.

The second factor is the development of antibody in response to the antigen inoculated in the form of vaccine. Again, as in the studies referred to above, this usually occurs between the 14th and 20th days in the course of vaccine treatment. From the potency of the vaccine and the ability of the patient to produce antibody are the limiting factors.

The third factor is the antigen possibly arising from the multiplication of the street virus introduced at the time of the bite. Obviously, there is...
no way of telling (except in those persons in whom the treatment fails and deaths from rabies ensue) when individuals received sufficient exposure for the street virus to start multiplying, nor when that multiplication ceased in any individual or what amount of virus was produced during the period of multiplication. With patients who subsequently die of rabies we can be fairly certain that relatively large amounts of street virus antigen appeared late in the incubation period. This antigen may call forth an antibody response just as effectively or even more effectively than that introduced in the vaccine.

Since in the serum-treated group all three and in the vaccine-treated group two of these factors may be operating simultaneously, it is difficult to interpret the meaning of the presence of antibody, particularly after the 1st day. It is possible to distinguish late in the course of treatment between antibody produced as a response to vaccine and antibody resulting from response to multiplying street virus. This applies in comparing the results in patients C1, C2, and C4. It might be speculated that C1 and C4, being fatal cases, had higher antibody levels because of the presence of more antigens, coming from multiplying street virus, whereas C2 had little such multiplication and responded to the antigen in the vaccine only. A further complicating factor is the known fact that certain individuals produce antibody very poorly or not at all in response to any type of antigenic stimulus. This would appear to be the case in patients C3 and C6, in which the difference possibly was that C6 had received an infectious dose of street virus at the time of the test while C5 had not.

Immunologically, the results in series A and B, where serum and vaccine were used, would seem more clear-cut and logical than those in series C. Here all twelve patients had passive antibody early in the course of treatment and all developed antibody later in the course of treatment, apparently as a response to vaccine, with the exception of one individual. This individual, the only fatal in the series, died before antibody development to vaccine could be expected in the light of the results obtained in the series receiving vaccine alone.

The question of the potency of the vaccine used in this clinical trial requires some discussion. From the antibody response in the patients in all series at the end of the treatment period it would appear that the vaccine was antigenic in humans. Although the potency test in mice showed a lower level of protection than 1000 LD<sub>50</sub>, which is usually considered the minimum requirement, this was in a test carried out three months after the actual use of the vaccine, at which time in usual practice the vaccine would have been at least two months beyond its expiration date. In probability a vaccine such as this, which had a potency of 500 LD<sub>50</sub> in November, would have had a greater potency had it been tested in the preceding August when it was used for treatment. In any event, the antibody response noted in the present series is somewhat analogous to that evolved in normal human subjects when a vaccine of higher potency was used.

RESUME

L'étude clinique des résultats du traitement au moyen du sérum et du vaccin antirabiques montrait que, dans les séries A, B et C, l'incidence de la maladie était la même. Les patients qui reçurent l'antiserum renflouaient dans les suites des bissaux, préservé à intervalles donnés au cours du traitement, afin d'évaluer le taux d'antigène et l'éclosion de la maladie.

Dans le groupe A, ayant reçu deux doses de 1er et de 4e jour et le vaccin donné 21 jours après, tous les patients présentaient un certain nombre d'anticorps dès les cinq premiers jours. Ce phénomène était moins visible jusqu'au 21<sup>er</sup> jour chez tous les individus. Il a généralement baissé à partir de ce moment jusqu'au 35<sup>er</sup> jour, suivi d'un taux qui a gardé un niveau de protéines élevée jusqu'à la fin de la période d'observation.

Dans le groupe B, ayant reçu une injection de 1er et du 7e jour et le vaccin donné 21 jours après, tous les patients présentaient un certain nombre d'anticorps dès les cinq premiers jours. Ce phénomène s'intensifiait jusqu'au 21<sup>er</sup> jour, chez les patients du 2e blessé de la série. L'incidence de la maladie était plus basse chez les patients du 1er blessé de la série.

Dans le groupe C, qui a reçu le vaccin, la présence d'antigène ne fut décelée qu'au 14<sup>er</sup> jour. Des traces infimes ont été observées jusqu'au 21<sup>er</sup> jour, suivi d'un certain nombre d'anticorps dans la série. L'incidence de la maladie était beaucoup plus basse que dans les deux autres séries.

Les résultats de ce travail n'ont pas de valeur théorique. Tous les patients traités par vaccin et sérum présentaient des résultats comparables avec les immunisations chimiques. L'administration des vaccins est faite en fonction de la durée de l'exposition à la maladie.

Il est donc possible de déterminer, au-delà du 21<sup>er</sup> jour, les indications de la maladie.

Les résultats de ce travail sont très utiles et largement explicable. Tous les patients traités par vaccin et sérum présentaient des résultats comparables avec les immunisations chimiques. L'administration des vaccins est faite en fonction de la durée de l'exposition à la maladie. L'incidence de la maladie est beaucoup plus basse que dans les deux autres séries.

Il est donc possible de déterminer, au-delà du 21<sup>er</sup> jour, les indications de la maladie.

* Further details will be included in a third paper of this series to appear shortly in this Bulletin.
no way of knowing (except in those persons in whose treatment fevers and deaths from rabies occurred) when individuals received sufficient exposure for the virus to start multiplying, nor when these multiplication occurred in any individual or what amount of virus was produced during the period of multiplication. With patients who subsequently died of rabies we can be fairly certain that relatively large amounts of virus antigen present late in the incubation period. This antigen may call forth an antibody response just as effectively as—or even more effectively than—that introduced in the vaccine.

Since in the vaccine-treated groups all three and in the vaccine-treated groups two of these factors may be operating simultaneously, it is difficult to interpret the meaning of the presence of antibody, particularly after the 21st day. It is impossible to distinguish late in the course of treatment between antibody produced as a response to vaccine and antibody resulting from response to multiplying street virus. This applies in comparing the results in patients C1, C2, and C4. It might be speculated that C1 and C4, being fatal cases, had higher antibody levels because of the presence of more antigen, coming from multiplying street virus, whereas C2 had little or no multiplication and responded to the antigen in the vaccine only. A further complicating factor is the known fact that certain individuals produce antibody very poorly or not at all in response to any type of antigenic stimulus. This would appear to be the case in patients C3 and C5, in whom the difference was possibly not that C3 had received an infectious dose of street virus at the time of the test while C5 had not.

Immunologically, the results in series A and B, where serum and vaccine were used, would seem more clear-cut and logical than those in series C. Here all twelve patients had passive antibody early in the course of treatment and developed antibody later in the course of treatment, apparently as a response to vaccine, with the exception of one individual. This individual, the only fatality in the series, died before antibody development to vaccine could be expected in the light of the results obtained in the series receiving vaccine alone.

The question of the potency of the vaccine used in this clinical trial requires some discussion. From the antibody response in the patients in all groups at the end of the treatment period it would appear that the vaccine was of high potency in humans. Although the potency test in mice showed a lower level of protection than 1000 LD₅₀, which is usually considered the minimum requirement, this was a test carried out three months after the normal use of the vaccine, in which time in commercial practice the vaccine would have been at least two months beyond its expiration date. In all probability a vaccine such as this, which had a potency of 531 LD₅₀ in November, would have had a greater potency had it been tested in the preceding August when it was used for treatment. In any event, the antibody response noted in the present series is somewhat analogous to that evoked in normal human subjects when a vaccine of higher potency was used.

**RESUME**

L'étude clinique des résultats des traitements, au moyen du sérum et du vaccin antirabiques, des sujets moribonds à la fin de l'année, a abouti à des résultats satisfaisants. Les sujets ont été vaccinés par injection intramusculaire dans les muscles des bras, précocement ou tardivement au cours du traitement, puis d'études ont été effectuées sur les rapports entre l'absence et l'absorption de l'antidote. Dans le groupe A, ayant reçu des traitements allant de 1er et 2e mois et au vaccin 21 jours, tout le monde présentait un certain nombre d'antigènes des cinq premiers jours. Ce nouveau 1er traitement, jusqu'à 24e jour chez tous les individus. Il a généralement basé par la route, après 24e jour, avant de recevoir un traitement antirabique initial à la fin du 2e mois. Tous les vaccins avaient rempli la fonction de traitement.

Dans le groupe B, ayant reçu un traitement de 1er et 2e mois et le vaccin avant 21 jours, on a observé un certain nombre d'antigènes de cinq premiers jours. De même, dans le group C, il a été administré 21 jours, chez 4 de 5, niveaux élevés. Les deux méthodes ont montré une certaine absence d'antigènes au-delà du 2e mois.

Dans le groupe C, qui a reçu le vaccin, la présence de l'antidote, qui ne dépassait pas 21 jours. Des trois sexes, âgé de 21 à 24e jour, propageant un certain nombre d'antigènes, de manière inconstante, une des deux méthodes a montré une certaine absence d'antigènes au-delà de la période d'observation considérée.

Les résultats cliniques montrent que diverses conditions immunitaires se développent au cours de la vaccination, et que des divers vaccins ou antidotes possibles, un vaccin ou antidote, peuvent être utilisés par le médecin, qui a apparemment bien le temps de le garer au-delà de la vaccination. Il peut être possible de déterminer, à partir de 21 jour, si le patient est plus ou moins sensible au vaccin.

Les résultats cliniques montrent que le vaccin est assez actif et largement applicable. Tous les patients ayant reçu le vaccin présentent des anticorps naturels ou des protéines étrangères trouvées dans les seringues, conformément à la méthode de l'administration de vaccin. L'absence de fièvre, le 1er mois, après que des jours de vaccination, est aussi due que des traces d'antigènes restant et que les anticorps actifs ne sont pas encore décelables dans le sérum.

Il est certain que les résultats de ce travail sont un exemple du vaccin, est ainsi efficace que le vaccin est idéal pour combattre les effets des morsures provoques à la vie.