PRELIMINARY OBSERVATIONS IN PRIMARY ANTIRABIES IMMUNIZATION OF MAN WITH DIFFERENT TYPES OF HIGH-EGG-PASSAGE FLURY VIRUS

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SYNOPSIS

High-egg-passage (HEP) Flury rabies vaccines, with a suckling-mouse LD_{50} titre of $10^{6.0}$ or more per ml, were used for primary immunization of 149 individuals showing no rabies antibodies in their pre-vaccination sera. An antibody response was elicited in 142, or 95%, of the total. A centrifuged vaccine reduced side reactions to a minimum. This same vaccine induced an antibody response in all of 79 individuals vaccinated. Furthermore, the antibody titre in this latter group approached that obtained with three injections of Semple-type vaccine.

In previous studies, Fox and his colleagues²,⁵ (and see page 869 of this issue) have demonstrated not only the safety but also the feasibility of employing a live, attenuated strain of rabies virus (high-egg-passage (HEP) Flury) to induce the production or mobilization of rabies antibodies in man. Several factors, including poor antibody response and some disagreeable reactions, occurred and required further clarification before HEP Flury rabies vaccine could be substituted for the conventional vaccines in current use for the prevention of rabies in human beings. However, these factors did not seem insuperable and Fox recommended that continued effort be made to improve the vaccine and to explore various dosage schedules of administration.

It seems timely, therefore, to report some of the clinical observations which were made on the use of improved vaccine containing the Flury strain of rabies virus attenuated by numerous passages through the chick embryo.
Methods and Materials

HEP Flury rabies centrifuged vaccine was prepared from chick embryos that were infected on the 7th and harvested on the 16th day of incubation. After homogenization of the harvested embryos (100% suspension) and centrifugation at approximately 900 × g for 20 minutes, the supernatant fluid was diluted to 70% original embryo suspension with casein digest solution, and freeze-dried. The vaccine was prepared for use by resuspending the dried product in saline (0.85%) or distilled water to a volume equivalent to 35% tissue concentration. Titres in suckling mice varied from 10^{6.2} to 10^{6.7} LD_{50} per ml; 0.02-ml quantities were used for intracerebral inoculation. There was no measureable loss in titre after storage for 18 months.

HEP Flury young-embryo and HEP Flury filtered vaccines were prepared as described by Fox et al. (see page 871 of this issue).

To compare more specifically three different types of HEP Flury vaccine in human beings only one method of administration was employed. Three intracutaneous injections of 0.2 ml were made at intervals of 5-7 days, on the volar surface of the forearm in subjects who had had no known previous contact with rabies antigen, and a single such injection was given to people who had had a previous course of rabies vaccine regardless of type. Usually, one of us (JMR) administered the first injection, whereas the second and third injections were administered by the subject's physician.

A serum specimen was taken from every volunteer before vaccination, and another during the second month after vaccination. Quantitative data on the effect of a single "booster" inoculation in individuals who had previously received vaccine will be reported at a later date.

Using the CVS rabbit-fixed strain of rabies virus, we screened all sera for the presence of antibodies. A mixture containing 9 volumes of unheated serum and 1 volume of virus (100-300 LD_{50} per mouse inoculum of the undiluted mixture) was incubated for 180 minutes at 37°C. The undiluted mixture and a 1/25 dilution in saline were each inoculated intracerebrally into 5 mice. The pre- and post-vaccination sera from each individual were always paired in the same test.

Quantitative determination of antibody concentration in the sera was done in the usual manner. A constant amount of virus (70-300 LD_{50} per mouse inoculum) was added to serial twofold dilutions of serum in saline, and the mixture was incubated for 90 minutes at 37°C. Five mice per serum-dilution-virus mixture were inoculated intracerebrally, and were observed for 15 days. The 50% end-points were then calculated by the Reed-Muench method. Appropriate controls were always included in each test.
Observations

The clinical studies of these vaccines were made primarily among voluntary groups of veterinarians, animal attendants, and speleologists, in widely separated areas in the USA. The results are summarized in Table I.

### TABLE I. RESULTS OF SCREENING TEST FOR PRESENCE OF ANTIBODIES FOLLOWING VACCINATION WITH HEP FLURY RABIES VACCINE

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccine Description</th>
<th>titre (LD&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
<th>Total persons</th>
<th>R *</th>
<th>SI R **</th>
<th>N †</th>
<th>% (R + SI R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Young embryo (inoculated at 3 days, harvested at 10 days)</td>
<td>5.1 ++</td>
<td>52</td>
<td>23</td>
<td>10</td>
<td>19</td>
<td>63.5</td>
</tr>
<tr>
<td>2</td>
<td>Young embryo</td>
<td>6.9</td>
<td>13</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Centrifuged</td>
<td>6.2</td>
<td>79</td>
<td>78</td>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>Young embryo</td>
<td>6.2</td>
<td>32</td>
<td>30</td>
<td>1</td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>5</td>
<td>Centrifuged</td>
<td>6.1</td>
<td>25</td>
<td>16</td>
<td>3</td>
<td>6</td>
<td>76</td>
</tr>
</tbody>
</table>

Three inoculations were given intracutaneously at 5- to 7-day intervals.

* R = 8 or more mice surviving out of 10.

** SI R = Approximately twice as many mice lived on the post-vaccinal as on the pre-vaccinal serum. This is interpreted as a borderline response.

† N = No difference between pre-vaccinal and post-vaccinal sera

++ Negative log with base 10

The vaccine administered to individuals in group 1 had a titre of 10<sup>5.1</sup> per ml for suckling mice. An antibody response was detectable in 33, or 63% of the total, but in 10 of these individuals the response was minimal. However, in groups 2-5 inclusive, the vaccines had a titre of 10<sup>6.0</sup> or more per ml. This is approximately 10 times the amount of virus present in the group-1 vaccine. Of 149 individuals in these groups, 142, or 95%, showed a detectable antibody response, and only 6 of these indicated a minimal response.

The most interesting data were obtained in group 3, where only centrifuged vaccine from two different lots was used. The screen test showed that all individuals developed antibodies, 78 with a good response, and only one with a minimal, or borderline, response. From these 78 sera, 46 were chosen at random for determination of antibody titre. These data are presented in Table II. Of the 46 sera tested, 3 had no detectable antibodies
when diluted 1/4, 5 had titres in the 1/4 to 1/7 range, and all others were greater than 1/8. One had a titre greater than 1/128. Thirty-six, or 82%, were in the 1/8 to 1/127 range.

The incidence of reactions can only be estimated inasmuch as the record is dependent upon a follow-up report from the subject in a great majority of cases. Pruritus at the site of inoculation was observed fairly frequently with this vaccine—a reaction commonly noted when many other vaccines have been injected by this route. Erythema and oedema were noted as troublesome signs and symptoms in about 10% of the subjects; these findings were observed much more frequently in those who had received filtered vaccine than in those receiving centrifuged vaccine. Similarly, axillary lymphadenopathy and/or pain in the axilla were noted more frequently in subjects receiving the filtered vaccine. It should be added, however, that this symptom did not prevent any person from completing the course of vaccine. With one exception, no systemic reactions were noted following the intracutaneous injection of HEP Flury vaccine in this series. One veterinarian experienced diplopia for approximately one week. This symptom was first noted three weeks after the third injection, cleared spontaneously, and could not be explained by an ophthalmologist except on the basis of third-nerve neuritis, possibly of vaccine origin.

**Discussion**

From these results and those given by Fox et al. on page 873 of this issue, it is reasonably clear that the response to HEP Flury rabies vaccine is directly related to its virus content. Therefore, the preparation must be sufficiently stable to guarantee that its living virus content at the time of use is high. Since the virus is relatively unstable in dried embryo suspensions, a stabilizer has always been used in the commercial Flury rabies
vaccines for use in animals. The stabilizer was not added to the experimental vaccines used in the previous tests in man. Therefore, it is entirely possible that loss of virus before the vaccine was used was responsible for some of the poor results obtained previously.

While there is no conclusive proof that excess embryo tissue interferes with antibody production, the evidence is clear that good antibody response can be elicited without the presence of excess embryo tissue. This tissue can be removed by centrifugation, and by this procedure most, if not all, of the undesirable side effects can be avoided.

The vaccine prepared from young embryos is quite costly because only a small amount can be obtained from each egg. Since neither the virus content nor the elicited antibody response was superior, there would appear to be no reason at the present time to consider further this more expensive type of preparation.

Experiences in cattle vaccinated with HEP Flury virus \(^1, 3\) have failed to demonstrate good correlation between antibody titre and resistance to challenge with either street or fixed virus. Nevertheless, in the absence of any other determinable factor, the level of antibody remains as the only indication of possible resistance. The quantitative data show that three intracutaneous injections of centrifuged vaccine elicited an antibody titre comparable to that obtained by Fox after a similar number of subcutaneous injections of Semple vaccine.

While this report concerns the antibody response of only 201 persons who received three intracutaneous injections of vaccine, it should be recorded that more than 1000 subjects have now received HEP Flury vaccine of all types. This attests further to the safety of a live attenuated virus vaccine as an immunizing agent against rabies.

A diminution in the number of reactions likewise suggests that advances have been made in the further refinement of the vaccine. For instance, suppuration at the site of injection was a not infrequent occurrence in the initial reports of Fox and Koprowski; in this series, suppuration was not reported by any subject. Similarly, the incidence of painful swelling of the forearm was reported much less frequently following the use of centrifuged vaccine than in group 1 when the filtered vaccine was used. Whether the experience of diplopia by one volunteer represents an allergic reaction due to some component of the vaccine cannot be determined at this time. The relatively late appearance of this symptom and its complete disappearance without other sequelae suggest the possibility of an allergic reaction.

RÉSUMÉ

La protection contre la rage par le vaccin vivant atténué Flury HEP a été démontrée par plusieurs auteurs. Cependant, certains échecs dus à une réponse-anticorps insuffisante ou à des réactions secondaires fâcheuses demandaient à être expliqués. Les auteurs ont
étudié les conséquences de la vaccination par le virus Flury HEP sur quelque 200 personnes. Dans leurs conclusions, ils rappellent que la réponse-anticorps dépend de la quantité de virus injectée. Il faut donc que la stabilité du vaccin assure la présence d’une quantité suffisante de virus lors de l’injection. Il est possible que l’absence d’adjuvant stabilisateur dans les vaccins utilisés lors de précédentes expériences puisse expliquer quelques-uns des résultats décevants.

L’excès de tissu embryonnaire provenant de la culture du virus peut être éliminé par centrifugation, ce qui supprime, en tout ou partie, les réactions secondaires désagréables.

Chez le bétail vacciné par le virus Flury HEP, on n’a pas pu établir de rapport net entre le niveau des anticorps et la résistance au virus des rues ou au virus fixe. Toutefois, ce niveau reste la seule indication actuellement connue d’une éventuelle résistance. Trois injections intracutanées de vaccin Flury centrifugé ont provoqué un même titre d’anticorps que trois injections sous-cutanées de vaccin Semple.

Le vaccin Flury a été administré jusqu’à maintenant à plus de 1000 personnes, ce qui atteste son innocuité. La purification du vaccin a également diminué de beaucoup les réactions locales consécutives à l’injection.

REFERENCES