Antibody Response to a Human Diploid Cell Rabies Vaccine

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An experimentally killed rabies virus vaccine prepared in a human diploid cell strain (WI-38)—Wyeth rabies vaccine (WRV)—was used by various injection schedules in two separate studies to define more closely in human volunteer subjects an effective vaccination schedule for pre- or postexposure immunization, particularly for donors of rabies-hyperimmune plasma. To permit valid comparisons between our results and those of other workers, antibody levels achieved were expressed in terms of international units (IU) per milliliter of serum. Antibody response of previously nonvaccinated persons were only modest after a single dose of WRV, never reaching a level higher than 0.80 IU/ml over a 56-day testing period. Moreover, antibody was not detected at 0.16 IU/ml before the 14th day, either after a single dose or after two doses given 3 days apart. The best response followed four doses of WRV given within 4 weeks. This schedule resulted in the highest rate of seroconversion to the >6 IU/ml antibody level required of potential rabies-immune plasma donors. Giving the first vaccine dose in aluminum hydroxide diluent did not enhance the antibody response. There was a definite suggestion in the various injection schedules that higher and more sustained antibody levels were reached when the interval between the first and second vaccine doses was longest. The greater immunogenicity of WRV as compared with duck embryo vaccine was best demonstrated by the fact that a single booster dose of duck embryo vaccine to previously vaccinated individuals resulted in only a sevenfold antibody rise during the following 56 days, whereas a booster dose of WRV elicited a 69-fold rise. Al(OH)₃ in the first dose of WRV had no effect, but the enhancing effect of a longer interval between vaccine doses was noted once again; 20 of 20 subjects who received three doses of WRV with 4 weeks between doses developed good levels of rabies antibody, and 19 exceeded 6 IU/ml.

In previous communications, we reported the preparation of rabies-immune globulin of human origin (RIGH) and the determination of its optimal dosage in volunteer subjects actively immunized with duck embryo rabies vaccine (4, 8). Later, we described the properties of RIGH and pointed out among other details that the immune plasma pool used for fractionation must contain at least 6 to 8 international units (IU) of rabies antibody to yield RIGH of acceptable potency (3). It goes without saying that hyperimmunization is the only means of inducing this level of rabies antibody in prospective plasma donors.

The choice of rabies vaccines to be used in man for hyperimmunization is limited. Vaccines prepared from brain tissue of adult animals are liable to cause allergic postvaccination reactions of the meningoencephalitis or polyneuritis type (1, 5). In view of this, a vaccine prepared from rabies-infected duck embryo tissues, and inactivated with beta-propiolactone, was introduced in 1956 (9). Gradually, this vaccine came to replace nervous tissue vaccines in the U.S.A. and is at present the only available licensed vaccine for either pre- or postexposure immunization. Although this vaccine proved to be a great deal safer than those derived from nervous tissue, it presents a number of disadvantages in the hyperimmunization of potential rabies-immune plasma donors: (i) the great number of injections needed; (ii) the undue occurrence of localized reactions; and (iii) the small number of persons who develop the necessary level of rabies-neutralizing antibody, despite repeated booster injections.

A beta-propiolactone-inactivated rabies vaccine, prepared from virus grown in a culture of human diploid cells (WI-38), was shown to have a higher antigen index in mice than duck embryo vaccine and to be immunogenic in monkeys (10, 12, 13). Another human diploid cell vaccine was prepared by Wyeth Laboratories, Philadelphia, Pa., by inactivation and
disaggregation of the virion with tri-(n)-butyl phosphate instead of beta-propiolactone (H. Tint, M. B. Dobkin, and B. A. Rubin, submitted for publication). In preliminary studies in man, this vaccine induced a good booster response with a single dose in those previously vaccinated and an acceptable antibody response after two doses in the majority of those who were never vaccinated before. Reactions were minimal and only local (14).

This paper summarizes the serological results of studies with Wyeth vaccine, the object of which was to define more closely an effective vaccination schedule for pre- or postexposure immunization, particularly for donors of rabies-hyperimmune plasma. Careful clinical observation revealed no adverse reactions in these studies. A detailed description of the mild symptoms reported will be given elsewhere (J. C. Loofbourow, V. J. Cabasso, T. Y. Cooper, B. Smith, and H. Hughes, submitted for publication).

MATERIALS AND METHODS

A supply of rabies virus vaccine (WRV), inactivated (Wyeth) (experimental lot 372-A-00101), was obtained from Howard Tint, Wyeth Laboratories, Philadelphia, Pa. The vaccine consisted of an inactivated, concentrated, and vacuum-dried extract of human diploid cell strain WI-38 which had been infected with the PM strain of rabies virus. Inactivation of the virus had been carried out with 0.1% tri-(n)-butyl phosphate in the presence of 0.1% Tween 80. The vaccine was dispensed in 1-ml doses in disposable syringes (Tubex) and was reconstituted just before use with either sterile distilled water or distilled water containing 0.1% aluminum hydroxide (Al(OH), diluent). At the time it was used, it had a mean potency ratio of 10 against the U.S. reference vaccine 176, 178, or 179, obtained from the Bureau of Biologics (BOB), Food and Drug Administration, Rockville, Md.

Rabies vaccine, duck embryo origin (DEV), was obtained commercially from a lot well within the expiration date at the time of the study.

Clinical studies: study 1. The objective of the study was to compare the rabies antibody response of previously nonvaccinated persons with various schedules of immunization, using one to four doses of WRV. The study was conducted at the medical facility of the Arizona State Prison, Florence, Ariz. The study population consisted of informed and consenting adults who were 21 to 55 years old and in good health. The 50 subjects admitted to the study were selected from among those shown negative for hepatitis B antigen and with no history of sensitivity to antibiotics or other allergies.

Study 2. This study had two objectives: (i) to obtain a rapid measure of the relative antigenicity of single doses of DEV or WRV by comparing secondary immune responses of previously vaccinated volunteers, and (ii) to study the immunogenicity of three doses of WRV at an interval of 4 weeks between doses in previously nonvaccinated subjects. This schedule is adaptable to the vaccination of first-year students seeking pre-exposure immunization. The study was carried out at the Cowell Student Health Center and Hospital of the University of California at Davis with 40 informed and consenting adult volunteers, 20 to 50 years of age, of either sex and in good health.

Serum antibody titration. The antibody response of each subject was determined by measuring the rabies-neutralizing antibody in each serum specimen according to the procedure outlined by the BOB of the Food and Drug Administration for potency testing of antirabies serum. This procedure was carried out in Swiss Webster mice as previously described (4). The antibody content of each sample tested is reported in this paper as international units of rabies antibody per milliliter. Computation of the international units per milliliter is made possible by the inclusion in each test set of the U.S. standard antirabies serum which is supplied by the BOB in dried form. When reconstituted for use, this serum has a designated antibody content of 2 IU/ml. During the course of the studies being reported, the mean serum neutralization titer obtained by us with this serum was 1:100, the value used in our computations.

To ascertain the reliability of the mouse serum neutralization test results, arrangements were made with the Center for Disease Control (CDC), Atlanta, Ga., to test in parallel with us 51 coded samples obtained from study 2. These samples represented three serial bleedings from 17 volunteers who received single booster doses of either DEV or WRV. In terms of absolute titers, those of CDC were consistently two to fivefold higher than ours. These higher titers were also reflected in the CDC findings for the BOB standard serum, the mean value of which was 1:300 in their hands. However, the differences between the two laboratories became much smaller when titers were converted to international units per milliliter (Fig. 1). In fact, the two sets of results coincided remarkably.

![Fig. 1. Comparison of serum neutralization test results in two laboratories.](image-url)
well when so expressed, considering the low degree of precision of the mouse test.

RESULTS

Study 1. The 50 volunteers accepted in the study were randomly assigned to one of the ten groups detailed in Table 1. In all cases except group X, WRV was reconstituted in sterile distilled water. The subjects in group X received the first injection of vaccine (day 0) reconstituted in Al(OH)₃ diluent, and they received the later injections of vaccine reconstituted in distilled water. All injections were made intramuscularly in the deltoid area in 1-ml volumes.

On the days specified in Table 1, 10 ml of whole blood was withdrawn from each participant, and the sera recovered from the specimens were frozen and shipped to the laboratory for later antibody level determination.

All samples from the same individual were tested at one time. Prevaccination sera were tested in a dilution range starting at 1:2 (permitting detection of 0.04 IU/ml), whereas later specimens were tested in dilution ranges starting at 1:8, permitting estimations of antibody levels as low as 0.16 IU/ml.

At different times early in the study, four individuals withdrew from it because of various reasons, but none because of an undue reaction to the vaccine. One each of the four individuals had been assigned to group II, III, V, or VIII (Table 1).

The antibody responses of groups I to X are presented in Table 2 and in Fig. 2 to 4. Considering the lack of precision of the mouse test, fairly consistent results were obtained for the individual subjects of each group. Consequently, although the small size of the group precluded arriving at definitive conclusions concerning an optimal schedule of immunization, a number of valid observations can be made.

As expected from their history, all participants in the study were negative for rabies antibody before vaccination.

In evaluating the serological response to pre-exposure rabies vaccination, any measurable antibody level is considered to have conferred on the individual an effective degree of resistance to natural infection (2). In this respect, all but 2 of the 46 participants in study 1 responded to vaccination with WRV, regardless of the number of vaccine doses or the schedule of injection. One of the two who failed to respond at the 0.16 IU/ml level had received one dose of vaccine (group I, Fig. 2) and the other three vaccine doses (group VII, Fig. 3).

From the standpoint of hyperimmunization of plasma donors, however, it was clear that the antibody response was insufficient, either after a single dose of WRV (group I, Fig. 2) or after two doses given 3 days apart (groups II and VI, Fig. 2 and 3). It was also clear that a single dose of vaccine resulted in only a modest antibody response over a 56-day testing period (group I, Fig. 2).

The groups receiving two doses of vaccine (II, III, and IV, Fig. 2) responded to essentially the same level of rabies antibody. However, there was a suggestion that, even though the rise of antibody was slower, it reached a higher level and was more sustained when the interval

| Group No. | No. in group* | No. of vaccine doses | Day of vaccine injection and/or bleeding(s)* |
|-----------|--------------|---------------------|-------------------------------------------|
|           |              |                     | 0 1 3 7 14 28 56 70 84                   |
| I         | 5            | 1                   | S-V S S S S S S S                        |
| I         | 4            | 2                   | S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V |
| III       | 4            | 2                   | S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V |
| IV        | 5            | 2                   | S-V S S S S S S S                        |
| V         | 4            | 3                   | S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V |
| VI        | 5            | 3                   | S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V |
| VII       | 5            | 3                   | S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V |
| VIII      | 4            | 3                   | S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V |
| IX        | 5            | 4                   | S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V |
| X         | 5            | 4                   | S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V S-V |

* At the initiation of the study, each group consisted of five individuals.

**S-V, Blood sampling followed shortly by vaccine administration; S, blood sampling only.

In all cases except group X, each dose consisted of 1 ml of vaccine reconstituted in sterile distilled water. All injections were given intramuscularly in the deltoid area. Subjects in group X received on day 0 1 ml of vaccine reconstituted in Al(OH)₃ diluent, also intramuscularly. Later doses to members of this group were reconstituted in sterile distilled water.
### Table 2. WRV study 1: antibody response to various immunization schedules

| Group no. | Antibody response | Antibody content (IU/ml) at the specified day post-vaccination* | No. with ≥ 6 IU/ml |
|-----------|-------------------|---------------------------------------------------------------|-------------------|
|           |                   | 0     | 1     | 3     | 7     | 14    | 28    | 56    | 70    | 84    |         |
| I         | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-0.42 | 0.16-0.80 | 0.16-0.80 | 0.05  | 0.28  | 0/5     |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.04    | 0.05    | 0.28    |     |     |         |
| II        | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-1.28 | 0.18-4.00 | 0.28-3.20 | 0.86  | 0.98  | 0/4     |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.11    | 0.86    | 0.98    |     |     |         |
| III       | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-0.32 | 0.72-5.00 | 0.40-5.00 | 2.08  | 1.42  | 0/4     |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.08    | 0.05    | 0.82    |     |     |         |
| IV        | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-0.50 | 0.20-1.28 | 0.80-5.12 | 1.26-3.20 | 2.00  | 0/5     |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.09    | 0.55    | 1.82    |     |     |         |
| V         | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-2.04 | 0.30-16.00 | 1.28-20.24 | 3.57  |     | 1/4     |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.14    | 2.08    | 3.57    |     |     |         |
| VI        | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-0.20 | 0.30-1.62 | 0.64-16.00 | 0.64-12.76 | 1/5   |         |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.03    | 0.56    | 1.87    | 1.61  |     |         |
| VII       | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-0.24 | 0.16-6.32 | 0.16-10.24 | <0.16-7.60 | 1/5   |         |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.05    | 0.26    | 1.27    | 0.95  |     |         |
| VIII      | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-2.00 | 0.16-2.00 | 0.22-16.28 | 1.00-32.44 | 1.26->10.24 | 1/4 |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.13    | 0.15    | 1.32    | 4.75  | <2.40 |         |
| IX        | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-2.00 | 1.20-12.60 | 3.32-20.24 | 1.28-6.34 | 2.78  | 2/5     |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.31    | 4.22    | 6.40    | 2.78  |     |         |
| X*        | Range             | 0.04  | 0.16  | 0.16  | 0.16  | 0.16-1.00 | 0.16-3.80 | 0.64-2.50 | 0.50-2.50 | 1.29  | 0/5     |
|           | GM*              | <0.04 | <0.16 | <0.16 | <0.16 | 0.12    | 0.69    | 1.65    |     |     |         |

* Boxes denote day of vaccine injection.

* GM, Geometric mean titers. For computation, antibody levels <0.04 or <0.16 were considered as zero.

* Group X is identical to group IX except that the first vaccine dose was reconstituted in Al(OH)₃ diluent.
between the two vaccine doses was longest (group IV, Fig. 2). This suggestion came also from among the groups receiving three doses of vaccine (Fig. 3), where group VIII had the longest interval between doses one and two and reached the highest antibody levels. This was observed in study 2 also, in the groups receiving
the same immunization schedule as group VIII.

The best response in study 1 was that of group IX (four injections of WRV within a 4-week period) with antibody levels of 3 to 20 IU/ml in the 56-day bleedings, and with two of the five subjects responding with 6 or more IU/ml that we require of immune plasma donors. Group X, which received an identical schedule of four injections except for the first dose in Al(OH)₃ diluent, responded measurably less well. This difference could suggest that Al(OH)₃ acted more as inhibitor in this case than as enhancer of the immune response, but as will be seen in study 2, this was most probably a chance occurrence due to the small number of participants in each group.

Finally, to afford a direct comparison of the response which followed four doses of WRV (group IX) to that elicited by 16 doses of DEV (14 daily doses plus booster doses on days 10 and 20 after the last daily dose), our findings in eight volunteers from a previous study (4) were plotted in Fig. 4 after conversion to international units per milliliter. It is seen that even though antibody levels rose to a higher level with 14 DEV doses during the first 3 weeks, this level was later surpassed by group IX after administration of the third and fourth doses of WRV. The highest geometric mean antibody level achieved with DEV was about 3 IU/ml, whereas that attained in group IX was 6.40 IU/ml.

Study 2. This study consisted of the following two parts.

(i) Booster effects of WRV and DEV. In the first part of the study, 20 persons who had received rabies vaccine earlier, but not during the previous 6 months, were assigned randomly to two groups of 10 each. Those in one group received one booster dose of WRV reconstituted in distilled water, whereas those in the other group were given one dose of DEV. On the days specified in Tables 3 and 4, 10 ml of blood was withdrawn from each subject, and the serum was processed and tested as described for study 1.

The antibody responses are presented in Tables 3 and 4 and Fig. 5. The randomness of the distribution of subjects between the two groups is supported by the antibody status of the participants before they received the booster dose of vaccine. In each group, only 4 of the 10 had measurable serum antibody levels (>0.04 IU/ml). The geometric mean antibody level was 0.05 IU/ml in the WRV group and 0.07 IU/ml in the DEV group. The highest antibody responses occurred at 7 to 28 days for both groups, depending upon the individual subject.

Among the ten subjects receiving DEV, five achieved an antibody level of 1 IU/ml or higher in one or more post-booster samples, but only one of the five reached a level of 6 IU/ml or higher, and this in only one of the four post-booster sera tested. In contrast, nine of the ten subjects who were given WRV attained antibody levels of 1 IU/ml or higher in one or more post-booster samples, and five of the nine reached levels of 6 IU/ml or higher in one or more of the post-booster sera. The greater immunogenic effect of WRV can best be summarized by the geometric mean antibody levels achieved by the two groups of volunteers: 0.90 to 3.40 IU/ml on the 7th through 56th days after WRV as against 0.32 to 0.48 IU/ml after DEV during the same period.

(ii) Responses of previously nonvaccinated subjects to three doses of WRV. In the second portion of this study, previously nonvaccinated subjects were randomly assigned to one of two groups. Each member of one group received three doses of WRV reconstituted in distilled water at 28-day intervals (Table 5). Each member of the other group was given three doses of WRV; the first was reconstituted in Al(OH)₃ diluent, and the last two were reconstituted in distilled water (Table 6). On the days specified in the tables, blood was drawn and the serum was processed as described above.

The antibody responses of the subjects are shown in Tables 5 and 6 and Fig. 6. All participants were free of measurable rabies antibody at the start of the study. The antibody responses were very similar in the two groups. After one dose of vaccine in distilled water, 7 of 11 subjects developed more than 1 IU/ml of
Table 5. WRV study 2: antibody response of previously unvaccinated volunteers after three doses (1) of Wyeth vaccinea

| Subject | Antibody (IU/ml)a after initial vaccine dose on day: |
|---------|-----------------------------------------------------|
|         | 0 (1) | 14 | 28 (1) | 56 (1) | 70 | 84 |
| 1       | <0.04 | <0.16 | <0.32 | 2.00 | 6.44 | 5.10 |
| 2       | <0.04 | 0.16 | 0.64 | 5.02 | 2.52 | 5.12 |
| 3       | <0.04 | 0.26 | 1.28 | 0.48 | 7.29 | 5.12 |
| 4       | <0.04 | 0.36 | 3.16 | 25.30 | 5.12 | 5.12 |
| 5       | <0.04 | 0.40 | 0.64 | 1.60 | 4.00 | 6.40 |
| 6       | <0.04 | 0.40 | 1.62 | 12.80 | 10.12 | 10.12 |
| 7       | <0.04 | 0.78 | 1.60 | 7.92 | 5.12 | 5.12 |
| 8       | <0.04 | 1.00 | 1.92 | 2.54 | 8.14 | 8.14 |
| 9       | <0.04 | 1.68 | 4.00 | 25.52 | 2.54 | 8.00 |
| 10      | <0.04 | 2.92 | 4.00 | 15.96 | 10.12 | 8.00 |
| 11      | <0.04 | 0.60 | 1.60 | 5.34 | 6.76 | 7.96 |

Geometric meanc

|         | 0.60 | 1.60 | 5.34 | 6.76 | 7.96 |

a All vaccine doses were reconstituted in distilled water and given intramuscularly in 1-ml amounts.

b See Table 3.

c See Table 3.

Table 6. WRV study 2: antibody response of previously unvaccinated volunteers after three doses (1) of Wyeth vaccinea

| Subject | Antibody (IU/ml)a after initial vaccine dose on day: |
|---------|-----------------------------------------------------|
|         | 0 (1) | 14 | 28 (1) | 56 (1) | 70 | 84 |
| 12      | <0.04 | <0.16 | 2.44 | 5.08 | 8.00 | 6.40 |
| 13      | <0.04 | 0.16 | <0.32 | 1.28 | 8.00 | 2.54 |
| 14      | <0.04 | 0.16 | 0.40 | 1.58 | 6.32 | 6.32 |
| 15      | <0.04 | 0.32 | 0.80 | 6.32 | 8.00 | 8.00 |
| 16      | <0.04 | 0.32 | 1.28 | 6.42 | 20.28 | 8.04 |
| 17      | <0.04 | 1.00 | 1.00 | 9.96 | 8.00 | 10.12 |
| 18      | <0.04 | 1.28 | 0.64 | 1.62 | 12.74 | 16.04 |
| 19      | <0.04 | 1.58 | 0.80 | 12.62 | 10.26 | 16.26 |
| 20      | <0.04 | 5.50 | 3.22 | 20.18 | 10.12 | 1.00 |

Geometric meanc

|         | 0.64 | 1.06 | 5.24 | 9.56 | 6.36 |

a The first vaccine dose was reconstituted in Al(OH)3 diluent. The second and third doses were reconstituted in distilled water. All injections were given intramuscularly in 1-ml amounts.

b See Table 3.

c See Table 3.

serum, and of those receiving the first dose in Al(OH)3 diluent, 4 of 9 showed this response. After two doses of vaccine, 10 of 11 subjects who received vaccine in distilled water and all 9 who received the first dose of vaccine in Al(OH)3 diluent exceeded the 1 IU/ml level. Subsequent bleedings showed that all participants had responded to levels higher than 1 IU/ml. The highest level (25 IU/ml) was achieved by two of them. The geometric mean values in the tables depict well the similarity of responses to the two schedules of vaccination, ruling out any beneficial contribution by Al(OH)3.

As in Fig. 4, the responses which followed 16 doses of DEV in a previous study (4) were also plotted in Fig. 6 for the purpose of comparison. As was noted earlier, the response to DEV was higher than that elicited by one dose of WRV during the first 4 weeks, but it was overtaken rapidly after the second and third doses of WRV. Highest geometric mean antibody levels achieved were 3 IU/ml of serum for DEV and 9.56 IU/ml for WRV.

**DISCUSSION**

The precision of results of serum antibody titrations in mice is generally considered to be low. In terms of absolute titers, the differences obtained on the same samples in our laboratory and at CDC are a case in point. It is difficult,
therefore, if not impossible, to make accurate comparisons from one test to another in the same laboratory or from one laboratory to another. The inaccuracy of the comparison becomes even greater when different laboratories use different test systems to determine the levels of rabies antibody. For example, Wiktor et al. (14) attempted to compare the immune response they obtained to one booster dose of WRV in a group of previously vaccinated subjects with that reported by others (3) for a group of previously nonvaccinated individuals after a complete series of DEV injections. They contrasted the 1:2,000 geometric mean antibody titer reached in 35 days by the WRV group, as measured by the plaque reduction technique in agarose-suspended baby hamster kidney cells (BHK 21/135), to the 1:145 geometric mean antibody titer achieved by the DEV group 40 days after initiation of the vaccination series, but measured by a serum neutralization test in mice. The conditions in these two studies were too different for a valid conclusion to be derived from this comparison.

Comparison of results among laboratories can be made more meaningful by inclusion of a defined reference antibody preparation in each laboratory and in each test, regardless of the test system. This can even be more meaningful if absolute titer levels are henceforth converted to a defined antibody unit value, as is available for the Standard Antirabies Serum available from the Public Health Service or from the World Health Organization. By this procedure, we were able to demonstrate a remarkably good agreement between our results and those obtained at CDC, despite the differences in absolute antibody titers. This consistency is also borne out by the results shown in the tables and figures of this report obtained over several months of testing. For the sake of more valid interpretation of results among rabies workers throughout the world, we urge that rabies antibody levels be henceforth expressed by all in terms of international units per milliliter of serum.

Turning to the relative effects of WRV and DEV, the results of our study demonstrate unequivocally the greater immunogenicity of the former. This was best illustrated by the secondary immune response of those previously given rabies vaccine. Starting with essentially the same pre-booster antibody level, the WRV group experienced a 69-fold antibody rise on the 28th day after the booster, whereas the highest antibody rise was only sevenfold in the DEV group on the 14th day.

A prompt antibody induction is mandatory in individuals exposed to rabies. When vaccination alone is considered, this may pose special if not insurmountable problems. Stavitsky (11) found in rabbits that the induction period for serum antibody ranges between 3 and 7 days, depending on the nature of the antigen, the site of antigen injection, and the presence of adjuvant which could enhance the proliferation of reticulum cells. Studies as systematic as this have not been carried out with rabies vaccine in man, but a number of workers have reported that after daily doses of nervous tissue or duck embryo vaccine, the earliest antibody detection occurred 6 to 8 days after the initial dose, and this only in a small percentage of recipients (2, 4, 6, 7). Whether more potent rabies vaccines or different injection schedules could materially shorten the induction time remains to be determined. However, a detectable level of circulating antibody can be afforded within 24 h after injection of an appropriate quantity of rabies immune globulin of human origin (4).

Where rapidity of active antibody induction is not critical, as in pre-exposure immunization, or when hyperimmunization of prospective donors of rabies immune plasma is carried out, it seems that a schedule of two or three doses of a vaccine having the potency of the WRV used in our studies can be very effective, although three doses may be required for hyperimmunization. In study 2, three doses of WRV induced circulating antibody in all 20 recipients, regardless of whether the first vaccine dose was reconstituted in water or in Al(OH)₃ diluent (Tables 5 and 6). In fact, in 19 of the 20 recipients, the antibody reached the level we require of potential rabies immune plasma donors, i.e., >6 IU/ml of serum. Thus, three vaccine doses spaced 4
weeks apart appeared to result in higher antibody levels than when three or four doses were given within shorter intervals (Table 2). As regards the effect of Al(OH)₃, the conditions of its use in our studies may not have been optimal.

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