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Rabies Neutralizing Antibody Response to Different Schedules of Serum and Vaccine Inoculations in Non-Exposed Persons: Part 3 *

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This study is the third in a series on virus-neutralizing antibody response to different schedules of antirabies serum and vaccines in previously non-exposed persons. Three types of vaccine were studied—phenolized (Semple), duck embryo and high-egg-passage (HEP) chicken embryo. Reduced schedules of vaccine, consisting of 2-7 inoculations given at various intervals, did not give results comparable in efficacy (time of appearance, level and persistence of antibody) with schedules comprising at least 14 daily inoculations of vaccine as determined in previous trials. The effectiveness of a booster dose in previously sensitized individuals was confirmed with a demonstration that a rise in serum antibody appears between 4 and 8 days after the booster inoculation. Effective sensitization appears to be as much a function of spacing of inoculations as of total dosage of vaccine antigen. Interference by immune serum with the antigenicity of subsequently administered vaccine, noted previously by the present authors and by other workers, was again confirmed. This interference could be overcome by the administration of a sufficient amount of vaccine.

This article reports on the third of a series of trials, co-ordinated by WHO, to study the virus-neutralizing antibody response to different schedules of antirabies serum and vaccine inoculations in previously non-exposed persons. These trials were undertaken to obtain information on the possibilities of increasing the efficiency of protection, and of minimizing the number of inoculations required to achieve a response equal or superior to that obtained in current practice in rabies immunization.

In the first trials (Atanasiu et al., 1956) virus neutralizing antibody levels in the serum were studied over a 28-day period following the first inoculations of vaccine and serum. There was suggestive evidence that the passive antibodies conferred by immune serum interfered somewhat with the active antibody response produced by the vaccine. One dose of immune serum combined with 14 daily doses of vaccine produced substantial levels of serum antibody throughout the entire 28-day period of study. The second trials (Atanasiu et al., 1957) were designed to study further the interfering effect of

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serum on vaccine and to study antibody response produced by reducing the number of vaccine inoculations. The possibility of pre-exposure immunization [1] was also explored. The results of the second trials can be summarized as follows:

1. Fourteen daily inoculations of phenolized vaccine produced a superior antibody response to that obtained with 3 inoculations given 5 days apart.

2. Three intradermal inoculations of HEP Flury vaccine given 5 days apart gave a low level of antibody response, but these individuals responded efficiently by producing antibody to a " booster " dose of the same vaccine given 6 months later.

3. Administration of phenolized vaccine or of HEP Flury vaccine alone did not produce detectable antibody in most individuals until between the 10th and the 15th day after the first inoculation of the vaccine.

4. Passive antibody following inoculation of antirabies serum persisted in some individuals for as long as 42 days. Two inoculations of serum administered 5 days apart did not give levels of antibody higher than those obtained with one inoculation.

5. One inoculation of serum completely suppressed antibody response to 3 inoculations of Flury vaccine given intradermally 5 days apart, and also prevented the preparation of the individuals to respond to a later " booster " dose of this vaccine.

6. Three inoculations of phenolized vaccine given 5 days apart acted efficiently in producing antibody by the 60th day. However, the interfering action of one and two inoculations of serum was clearly defined in this schedule.

7. One inoculation of serum had no clearly demonstrated suppressive effect on the active antibody response to 14 daily doses of phenolized vaccine; two doses of serum given in the same combination definitely interfered with the production of an active antibody response.

The trials described in the present paper were arranged to investigate further possible pre-exposure immunization schedules, and various reduced schedules of inoculations of three different types of vaccine, with and without serum, to clarify problems of antibody response to primary courses as well as to booster inoculations of vaccine.

[1] This term is used to represent possible immunization procedures for special groups of individuals at risk of being exposed to rabies, e.g., laboratory workers, veterinarians, etc.

MATERIALS AND METHODS

Inoculation schedules

Adult male human volunteers with no known history of exposure to rabies or of having received rabies vaccine were divided into random groups. Serum samples were taken following vaccination according to the schedule given in Table 1. The following outlines the reasons for the different schedules used as shown in Table 1.

HEP vaccine. HEP vaccine was given to Groups 1 to 16,[2] which may be subdivided as follows.

(*a*) Groups 1 to 5 were designed to determine the optimum spacing of vaccine inoculations for pre-exposure immunization.

(*b*) Group 6A (with the Control Group 6) was designed to study the effect of serum given at the time of a booster dose of vaccine.

(*c*) Group 7 was designed to compare an HEP vaccination schedule, which seemed to elicit the best response in preceding trials, with the same schedule for two other vaccines, phenolized and duck embryo (Groups 17A and 18A).

(*d*) Groups 9 to 16 were devised to study more fully the response to different vaccine schedules following early serum therapy. Groups 11, 12 and 13 concerned the minimum number of vaccine inoculations which could be used when given at about the time of decline of passive antibodies from immune serum as determined in previous trials (Atanasiu et al., 1956, 1957). Groups 15 and 16, the control groups for 9 and 11, were set up because previous work had indicated a suppressive effect of serum on vaccine-produced antibodies when both were given on the same day. Group 14 was essentially a modification of the usual post-exposure treatment for comparison with the phenolized and duck embryo vaccines (Groups 27 and 28). Group 14A was the control for Group 14.

Duck embryo and phenolized vaccines. Groups 17 to 28 included several parallel schedules for these two vaccines; e.g., three doses plus a booster dose, with or without serum. Groups 17A and 18A were added to study the effect of a fourth dose being added to the three doses given at 5-day intervals. Group 23A included one serum dose on Day 1 for comparison with Group 23; sera were unavailable for comparative groups for 21 and 22. It will be

[2] For technical reasons Group 8 was dropped before the experiments were begun and is therefore not included in these schedules.

TABLE 1

SCHEDULES FOR VACCINE AND SERUM INOCULATIONS AND COLLECTION OF BLOOD SPECIMENS

Group No.	Type of vaccine and schedule of inoculations (days)	Serum given	Blood specimens taken on following days	No. of individuals in group
	HEP			
1	0, 30	Nil	0, 60	20
2	0, 10, 30	Nil	0, 20, 30, 60	20
3	0, 10, 60	Nil	0, 20, 60, 90	20
4	0, 10, 90	Nil	0, 20, 90, 120	20
5	0, 10, 120	Nil	0, 20, 120, 150	20
6	0, 5, 10, 90	Nil	0, 20, 60, 90, 92, 94, 98, 120	20
6A	0, 5, 10, 90	Day 91	0, 20, 60, 90, 92, 94, 98, 120	20
7	0, 5, 10, 15, 90	Nil	0, 20, 60, 90, 94, 120	20
9	0, 10, 30, 120	Day 1	0, 60, 120, 150	18
10	0, 10, 15, 120	Day 1	0, 60, 120, 150	18
11	0, 15, 20, 120	Day 1	0, 60, 120, 150	18
12	0, 15, 20, 25, 120	Day 1	0, 60, 120, 150	18
13	0, 15, 20, 25, 28, 30, 120	Day 1	0, 60, 120, 150	18
14	0, 2, 4, 6, 8, 10, 12, 120	Day 1	0, 60, 120, 150	18
14A	0, 2, 4, 6, 8, 10, 12, 120	Nil	0, 60, 120, 150	18
15	0, 10, 30, 120	Day 0	0, 60, 120, 150	18
16	0, 15, 20, 120	Day 0	0, 60, 120, 150	18
17	Phenolized 0, 5, 10, 90	Nil	0, 20, 60, 90, 92, 94, 98, 120, 150	20
17A	Phenolized 0, 5, 10, 15, 90	Nil	0, 15, 20, 60, 90, 92, 94, 98, 120, 150	10
18	Duck embryo 0, 5, 10, 90	Nil	0, 20, 60, 90, 92, 94, 98, 120, 150	10
18A	Duck embryo 0, 5, 10, 15, 90	Nil	0, 15, 20, 60, 90, 92, 94, 98, 120, 150	10
19	Phenolized 0, 5, 10, 90	Day 91	0, 20, 60, 90, 92, 94, 98, 120, 150	20
20	Duck embryo 0, 5, 10, 90	Day 91	0, 20, 60, 90, 92, 94, 98, 120, 150	10
21	Phenolized 0, 1, 2, 10	Nil	0, 10, 15, 30, 60	30
22	Duck embryo 0, 1, 2, 10	Nil	0, 10, 15, 30, 60	10
23	Phenolized 0, 1, 2, 10, 120	Nil	0, 10, 15, 30, 60, 120, 150	26
23A	Phenolized 0, 1, 2, 10, 120	Day 1	0, 10, 15, 30, 60, 120, 150	14
24	Duck embryo 0, 1, 2, 10, 120	Day 1	0, 10, 15, 30, 60, 120, 150	10
25	Phenolized 0, 2, 4, 6, 8, 10, 120	Nil	0, 10, 15, 30, 60, 120, 124, 150	20
26	Duck embryo 0, 2, 4, 6, 8, 10, 120	Nil	0, 10, 15, 30, 60, 120, 124, 150	10
27	Phenolized 0, 2, 4, 6, 8, 10, 120	Day 1	0, 10, 15, 30, 60, 120, 124, 150	20
28	Duck embryo 0, 2, 4, 6, 8, 10, 120	Day 1	0, 10, 15, 30, 60, 120, 124, 150	10
Total 32				Total 552

noticed that Groups 17 to 20 correspond to Groups 6, 6A and 7 in the HEP vaccine groups; similarly Groups 25 to 28 correspond to Groups 14 and 14A, except for one inoculation on Day 12.

Collection and despatch of test sera

The blood specimens, which totalled over 3000, were left at room temperature for 2 hours before being refrigerated. Because of conditions in the field in Northern Nigeria, where the trials were undertaken (Cannon, 1960), the serum was usually not separated from the clot until the specimens reached a central laboratory some 8 to 24 hours later. The serum specimens were then stored for several months at 4°C, but during this time several mechanical breakdowns in refrigeration occurred. Many serum

specimens, in addition to showing haemolysis, were found to be contaminated and were Seitz-filtered. The specimens were eventually despatched by air to the Institut Pasteur, Paris, where they were sorted out into groups and distributed to the following laboratories for testing:

1. Department of Tropical Medicine and Public Health, Tulane University, New Orleans, La., USA;
2. National Institutes of Health, Laboratory of Biology of Viruses, Bethesda, Md., USA;
3. United States Department of Health, Education, and Welfare, Communicable Disease Center, Virus Laboratory, Montgomery, Ala., USA;
4. The Wistar Institute, Philadelphia, Pa., USA;
5. Institut Pasteur, Service des Virus, Paris, France;
6. National School of Public Health, Virus Laboratory, Madrid, Spain;
7. Division of Laboratories and Research, State of New York Department of Health, Albany, N.Y., USA.

Vaccines

The high-egg-passage chicken embryo vaccine (HEP) [1] (Koprowski, 1954a) was a freeze-dried preparation of living Flury strain of rabies virus (Koprowski & Cox, 1948), which had had in succession 199 chick embryo passages, 82 Maitland-type tissue cultures and another 6 one-day-old chick embryo passages. The material containing living virus was rehydrated with sterile distilled water to constitute a 70% concentration of chick embryo tissue; 0.2 ml was injected intradermally in the deltoid region. The vaccine had satisfactorily passed the guinea-pig (Koprowski, 1954b) and the adult mouse potency tests used for this type of vaccine (WHO Expert Committee on Rabies, 1960) and showed no drop in potency when re-tested upon return from the field where the inoculations had taken place.

The duck embryo vaccine [2] was a freeze-dried preparation of embryonic duck tissue infected with fixed rabies virus and inactivated by 1: 3000 β-propiolactone (Peck et al., 1955). The potency test, NIH type (Kaplan, 1954), on this vaccine showed protection 3.42 times that of the NIH standard vaccine in use at that time. The vaccine was reconstituted with 1.1 ml distilled water to give a 10% tissue suspension, and this amount was inoculated subcutaneously in the flank region.

The phenolized vaccine,[3] Semple type, was a 4% suspension of sheep brain. 2.5 ml of the vaccine were inoculated subcutaneously into the flank. The Habel potency test (Habel, 1954b) showed $10^{4.5}$ LD_{50} protection before the vaccine was sent into the field for inoculation, and $10^{4.1}$ upon return from the field after the inoculations had been performed.

Antirabies serum

This was a concentrated horse serum [4] containing approximately 100 international units per ml, according to the potency test recommended for this preparation (WHO Expert Committee on Rabies, 1960). 4000 units (40 ml) were given to volunteers approximately 18 years of age or older estimated to weigh about 70 kg or more, and 2000-3000 units were given to younger and lighter individuals. Tests of the serum returned from the field showed no loss in potency.

Serum neutralization (SN) tests

The " regular " SN test as described in our previous reports (Atanasiu et al., 1956, 1957) was used for the serum specimens. The procedure consisted first of screening all serum specimens for detectable antibody at a 1: 2 final dilution of serum against about 50 LD_{50} of fixed (CVS) virus. Results of the test were sometimes inconsistent when less than 30 LD_{50} of virus were used in the test, but not when the amount of virus was increased to 300 LD_{50}. After incubation at 37°C for 1½ hours the serum-virus mixture was inoculated intracerebrally into five mice. Serum specimens which protected at least two out of the five mice were then re-tested in a more quantitative test, using fivefold final serum dilutions of 1: 5 through 1: 625 in mixture with virus.

The above procedure was finally adopted after further experience using the " modified " technique described in our second report (Atanasiu et al., 1957) gave variable results on the same sera tested in different laboratories. Thus while the " modified " technique, using 19 parts of serum to 1 part of virus, gave a few positive results where the 1: 2 dilution of the " regular " procedure was negative, this occurred inconsistently. Also, the " modified " technique required the use of too much serum from the limited quantity available, and in several instances a non-

[1] Kindly provided by Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y., USA.

[2] Kindly supplied by the Lilly Laboratory for Clinical Research, Indianapolis, Indiana, USA.

[3] Kindly supplied by the Rabies Vaccine Laboratory, Federal Laboratory Service, Yaba, Lagos, Nigeria.

[4] Kindly supplied by Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y., and the Institut Pasteur, Paris.

specific inhibitory effect on the virus occurred. Pre-inoculation specimens were always examined in the series of specimens from each individual, all of which were tested at the same time, and the series was discarded if the pre-inoculation specimen showed neutralizing capacity.

Where serum specimens showed gross contamination (mould) antibiotics (100 units each of penicillin and streptomycin and 50 units of mycostatin) were included in the suspending media of the serum-virus mixtures. The use of antibiotics was discarded later on since they seemed to exert some non-specific inhibitory effect on the virus. It is interesting to note that check titrations repeated on some 15 random samples of positive serum specimens 1½ years after the first tests were made (a total of 2½ years after the serum specimens were taken) showed identical end-point titres in the two tests performed in different laboratories. This suggests not only the stability of the rabies neutralizing antibody to relatively rough treatment in handling and storage (shipping, temperature changes, contamination), but also the reliability of comparing results of the qualitative and quantitative tests performed in the different laboratories.

The collaborating laboratories tested all available serum specimens from single individuals in each of the groups. Thus each of the laboratories examined all sera from two or three individuals from each of the groups given in Table 1.

Because of insufficient serum and contamination of specimens in some instances, not all of the specimens collected could be examined. Tables 2 to 6 show the total number of specimens which were examined in each group. Quantitative tests were performed on specimens positive to screening only where the additional tests were considered to be warranted by the additional information they might yield which would be of possible use in relation to vaccination schedules in the future.

RESULTS

Tables 2 to 6 group the results of the neutralization tests according to comparable schedules of the vaccines and serum. The heading " positive for antibody " in these tables refers to the ratio of subjects whose sera neutralized rabies virus regardless of the serum dilution employed in the test, i.e., those positive at the 1: 2 final dilution of serum used for screening purposes as well as those which showed higher end-points in subsequent quantitative tests. Tables 4 and 6 also include neutralizing end-point titre ranges of sera obtained in quantitative tests; these give evidence of superior or inferior antigenic action of one or the other schedules of vaccine, or of apparent interfering action of the antirabies serum affecting the antibody levels obtained. The results of quantitative examination have not been tabulated in all instances; but some of the non-tabulated data will be referred to, where indicated, in the analyses and discussion to follow.

Table 2: Antibody response to minimal dosage schedules of HEP vaccine

The ratio of individuals whose sera neutralized rabies virus was high in all groups tested when the interval between the two injections of the vaccine was 20 days or longer. For instance, in Group 2 only 4 out of 12 subjects developed antibody after two injections of vaccine given at 10 days' interval. However, another injection of vaccine given 20 days after the second dose raised considerably the number of positive responses to 10 out of 12 tested. The

TABLE 2

ANTIBODY RESPONSE TO MINIMAL DOSAGE SCHEDULES OF HEP VACCINE

Group No.	Type of vaccine and schedule of inoculations (days)	Serum given	Positive for antibody at various periods following first inoculation of vaccine					
			20 days	30 days	60 days	90 days	120 days	150 days
1	HEP: 0, 30	Nil			9/11			
2	HEP: 0, 10, 30	Nil	5/12	4/12	10/12			
3	HEP: 0, 10, 60	Nil	3/12		6/11	11/12		
4	HEP: 0, 10, 90	Nil	3/10			2/8	8/10	
5	HEP: 0, 10, 120	Nil	4/13				3/13	9/13

actual number of injections of vaccine preceding the last dose given more than 20 days after the preceding one was without effect on the final outcome of the immunization; for example, as many subjects in Group 1 receiving two injections of vaccine showed positive response as in the remaining groups when three injections of vaccine were given.

Table 3: Antibody response to various dosage schedules of HEP vaccine alone, and with serum given at the time of first inoculation of vaccine or one day later

Immune serum given simultaneously with the HEP vaccine (Groups 15 and 16) interfered with the development of actively produced antibody during 120 days following administration of the first dose of vaccine, and in one of the two Groups (Group 16) seemed to interfere with the booster dose of vaccine given on the 120th day. The suppressive action of immune serum to the formation of active antibodies from either primary or booster inoculations was less manifest when serum was given one day after the first injection of vaccine (Groups 9, 10, 11 and 12).

Table 4 : Comparison of reduced schedules using HEP, duck embryo and phenolized vaccine alone, and with serum given one day after the booster inoculation of vaccine

When either 3 or 4 primary doses of vaccine were given 5 days apart, the HEP vaccine elicited antibody responses in a larger number of subjects than the duck embryo and phenolized vaccines when blood specimens taken 60 and 90 days following the first inoculation of the vaccine were tested. Following the booster dose of vaccine at 90 days more HEP subjects likewise started to respond with antibody production 4-8 days after the booster dose. This ratio evened out during the rest of the 30 days following booster inoculation.

Serum given the day after the 90-day booster inoculation of vaccines seemed again to interfere with the booster effect, as evidenced by the lower ratio of positive responses among subjects bled on the 150th day (when passive antibodies could be discounted) in the serum Groups 19 and 20 in comparison to those which received vaccine alone in control Groups 17A and 18A. Moreover, quantitative tests (not tabulated) on some of the serum specimens revealed that none of the 57 post-booster specimens tested from individuals who had received serum achieved an antibody level range of 125-625, whereas 9 out of 67 specimens from those who did not receive serum achieved this level.

The results here show also that the presence of detectable antibody is useful, but not essential, to indicate that the individual has been immunogenically sensitized to respond to a later booster dose. Thus in groups not receiving serum (6, 7, 17, 17A, 18 and 18A) the Day 60 specimen showed that very few individuals given phenolized or duck embryo vaccine showed circulating antibody (1/21 for phenolized and 1/14 for duck embryo) as compared with HEP (15/25); yet 30 or 60 days following a booster dose, the responses to the three vaccines were similar (15/21 phenolized, 9/15 duck embryo, 18/28 HEP).

Table 5 : Comparison of reduced schedules using phenolized and duck embryo vaccines alone, and with serum given one day following the first inoculation of vaccine

Apparently neither the phenolized nor the duck embryo vaccine produced adequate antibody responses when given in the stated reduced dosage schedule (Groups 21, 22 and 23). However, again a vaccine booster effect was observed when a dose of phenolized vaccine was given 110 days after the last dose (Group 23). This booster effect (observed on the 150-day blood samples) was suppressed when immune serum was given one day after the first dose of vaccine (Group 23A).

The duration of passive immunity from serum can be seen in the 10-, 15- and 30-day specimens of Groups 23A and 24. Thirty-four of these specimens were tested quantitatively (not tabulated); 25 out of 34 had antibody end-points which fell within the 1: 5 to 1: 25 serum dilution range, and the remaining nine were in the 1: 25 to 1: 125 range.

Table 6 : Comparison of reduced schedules for possible post-exposure immunization using HEP, phenolized and duck embryo vaccines alone, and with serum given early in the schedule

The results of this series confirm observations summarized in Table 2, that an increase of HEP vaccine doses to 7 injections (Group 14A), given on alternate days, does not result in a better ratio of positive responses than when two injections are given (Group 1, Table 2). The booster effect of vaccine administered on the 120th day was again apparent. However, somewhat in contrast to the results obtained in the groups presented in Table 3, immune serum seemed to have had no interfering effect with the antibody responses elicited by the increased vaccination dosage of HEP (60-day specimens of Group 14A compared with Groups 13 and

TABLE 3

ANTIBODY RESPONSE TO VARIOUS DOSAGE SCHEDULES OF HEP VACCINE ALONE, AND WITH SERUM GIVEN AT THE TIME OF FIRST INOCULATION OF THE VACCINE OR ONE DAY LATER

Group No.	Type of vaccine and schedule of inoculations (days)	Serum given	Positive for antibody at various periods following first inoculation of vaccine				
			20 days	30 days	60 days	120 days	150 days
2	HEP: 0, 10, 30	Nil	5/12	4/12	10/12		
9	HEP: 0, 10, 30, 120	Day 1			6/9	4/10	5/8
15	HEP: 0, 10, 30, 120	Day 0			1/9	0/10	5/12
5	HEP: 0, 10, 120	Nil	4/13			3/13	9/13
10	HEP: 0, 10, 15, 120	Day 1			0/10	0/10	5/10
11	HEP: 0, 15, 20, 120	Day 1			3/10	1/12	8/12
16	HEP: 0, 15, 20, 120	Day 0			2/9	0/9	1/6
12	HEP: 0, 15, 20, 25, 120	Day 1			4/9	2/7	6/10

TABLE 4

COMPARISON OF REDUCED SCHEDULES USING HEP, DUCK EMBRYO AND PHENOLIZED VACCINE ALONE, AND WITH SERUM GIVEN ONE DAY AFTER THE BOOSTER INOCULATION OF VACCINE

Group No.	Type of vaccine and schedule of inoculations (days)	Serum given	Positive for antibody at various periods following first inoculation of vaccine									
			15 days	20 days	60 days	90 days	92 days	94 days	98 days	120 days	Antibody level [a]	150 days
6	HEP: 0, 5, 10, 90	Nil		1/14	3/15	4/13	4/13	7/16	13/17	7/13	1 : 2 (3) 1 : 5 (2) 1 : 25 (2)	
17	Phenolized: 0, 5, 10, 90	Nil		1/17	0/15	1/16	0/17	0/17	8/16	10/14	1 : 2 (3) 1 : 5 (2) 1 : 25 (4) 1 : 125 (1)	5/16
18	Duck embryo: 0, 5, 10, 90	Nil		0/6	1/8	0/7	0/7	1/8	5/8	3/8	1 : 2 (2) 1 : 125 (1)	2/7
7	HEP: 0, 5, 10, 15, 90	Nil		4/9	7/10	4/10		6/12		11/15	1 : 2 (1) 1 : 5 (7) 1 : 25 (3)	
17A	Phenolized: 0, 5, 10, 15, 90	Nil	1/6	1/6	1/6	1/4	1/7	2/5	4/7	5/7	1 : 2 (1) 1 : 25 (3) 1 : 125 (1)	4/7
18A	Duck embryo: 0, 5, 10, 15, 90	Nil	1/6	4/6	0/6	3/7	1/6	1/6	7/7	6/7	1 : 2 (2) 1 : 5 (1) 1 : 25 (3)	6/7
6A	HEP: 0, 5, 10, 90	Day 91		1/14	2/9	4/14	15/15	13/13	15/15	14/15	1 : 2 (5) 1 : 5 (6) 1 : 25 (3)	
19	Phenolized: 0, 5, 10, 90	Day 91		2/14	1/14	0/16	15/15	15/15	15/16	11/15	1 : 5 (10) 1 : 25 (1)	2/16
20	Duck embryo: 0, 5, 10, 90	Day 91		2/5	3/7	1/6	6/6	5/5	6/6	4/5	1 : 5 (3) 1 : 25 (1)	1/7

[a] This represents the lower figure of the fivefold serum dilution range used in the serum neutralization test. Thus 1 : 5 indicates hat the end-point fell between the 1 : 5 and 1 : 25 dilution of serum being tested. Figures in parentheses indicate the number of specimens reacting at the respective dilution.

TABLE 5

COMPARISON OF REDUCED SCHEDULE USING PHENOLIZED AND DUCK EMBRYO VACCINES ALONE, AND WITH SERUM GIVEN ONE DAY FOLLOWING THE FIRST INOCULATION OF VACCINE

Group No.	Type of vaccine and schedule of inoculations (days)	Serum given	Positive for antibody at various periods following first inoculation of vaccine					
			10 days	15 days	30 days	60 days	120 days	150 days
21	Phenolized: 0, 1, 2, 10	Nil	0/20	3/23	2/20	2/23		
22	Duck embryo: 0, 1, 2, 10	Nil	2/6	1/6	2/9	5/9		
23	Phenolized: 0, 1, 2, 10, 120	Nil	0/21	1/20	3/17	2/21	0/20	10/23
23A	Phenolized: 0, 1, 2, 10, 120	Day 1	9/9	9/9	7/8	0/9	0/9	1/10
24	Duck embryo: 0, 1, 2, 10, 120	Day 1	5/7	5/5	4/6	1/6	0/7	2/6

14), and the suppressive effect on the booster response to vaccine was also apparently eliminated by the increased number of inoculations of vaccine in the primary course.

The antibody responses to the 7 injections of duck embryo vaccine were poor (Group 26), and it is impossible to evaluate the role of immune serum in this type of treatment. However, administration of immune serum two days after the first dose of phenolized vaccine (Group 27) seemed to suppress

TABLE 6

COMPARISON OF REDUCED SCHEDULES FOR POSSIBLE POST-EXPOSURE IMMUNIZATIONS USING HEP, PHENOLIZED AND DUCK EMBRYO VACCINES ALONE, AND WITH SERUM GIVEN EARLY IN THE SCHEDULE

Group No.	Type of vaccine and schedule of inoculations (days)	Serum given	Positive for antibody at various periods following first inoculation of vaccine							
			10 days	15 days	30 days	60 days	120 days	124 days	150 days	Antibody level [a]
14A	HEP: 0, 2, 4, 6, 8, 10, 12,[b] 120	Nil				4/12	5/15		11/14	1 : 2 (1) 1 : 5 (2) 1 : 25 (5) 1 : 125 (3)
25	Phenolized: 0, 2, 4, 6, 8, 10, 120	Nil	1/14	2/13	3/13	0/12	2/14	3/15	13/16	1 : 2 (3) 1 : 5 (3) 1 : 25 (7)
26	Duck embryo: 0, 2, 4, 6, 8, 10, 120	Nil	1/6	1/7	2/7	1/4	1/7	2/7	3/7	1 : 5 (1) 1 : 5 (1) 1 : 125 (1)
27	Phenolized: 0, 2, 4, 6, 8, 10, 120	Day 2	10/11	10/11	7/9	1/11	0/12	1/13	4/13	1 : 2 (3) 1 : 5 (1)
28	Duck embryo: 0, 2, 4, 6, 8, 10, 120	Day 2	5/5	4/4	5/5	0/5	0/8	0/8	2/7	1 : 2 (2)
14	HEP: 0, 2, 4, 6, 8, 10, 12,[b] 120	Day 1				4/14	1/15		11/14	1 : 2 (3) 1 : 5 (3) 1 : 25 (5)
13	HEP: 0, 15, 20, 25, 28, 30, 120	Day 1				6/10	2/9		8/10	1 : 2 (4) 1 : 5 (1) 1 : 25 (3)

[a] This represents the lower figure of the fivefold serum dilution range used in the serum neutralization test. Thus 1 : 5 indicates that the end-point fell between the 1 : 5 and 1 : 25 dilution of serum being tested. Figures in parentheses indicate the number of specimens reacting at the respective dilution.

[b] Notice extra inoculation of HEP vaccine on day 12.

the effect of the booster dose of vaccine as compared with the control group receiving vaccine alone (Group 25).

The " reduced " phenolized vaccine schedule (Group 25) did not appear to give satisfactory results from the standpoint of its possible use as a post-exposure treatment, but there was no comparable group in the present series representing the standard 14 injections given at daily intervals. Response of Group 25 to a booster inoculation, however, was good.

DISCUSSION

The discussions of the first two reports on this series of experiments (Atanasiu et al., 1956, 1957) review the limitations of the experimental arrangement and the relationship and significance of neutralizing antibody to protection against rabies infection. These points, therefore, will not be further considered here. The trials described in the present paper confirm the validity of grouping and comparing the results of examination of serum specimens obtained in seven different laboratories where the same prescribed techniques were followed. In both qualitative and quantitative determinations for virus neutralizing antibody there was again a consistency between serial specimens in individuals, between individuals in each group, between groups receiving similar treatment, and between the three series of trials. This was further supported by independent checks on a group of serum specimens which gave identical results to those obtained on the same specimens in different laboratories over 18 months previously.

The main purposes of the present series were to investigate further possible immunization schedules, using various reduced schedules of inoculations of three different types of vaccine. Vaccine inoculations were carried out with and without serum to clarify problems of antibody response to primary courses as well as to booster inoculations of vaccine. The aim of these reduced schedules was (1) to minimize the difficulties now encountered in post-exposure treatment schedules where large numbers of inoculations are employed, and where paralytic accidents may occur from vaccines containing large amounts of nervous tissue; and (2) further to explore pre-exposure immunization.

The early presence and persistence of antibody, of sufficient level to be detected by the tests employed, will be the criteria for interpreting the results in terms of probable efficacy of protection to rabies exposure (see Discussion in first report (Atanasiu et al. 1956)). Because of the large number of serum specimens, the end-point titre of antibody was determined only for certain groups of specimens. The results and their implications may be summarized as follows.

Reduced number of vaccine inoculations for possible use as a primary course in post-exposure treatment

If individuals have never previously received rabies vaccine inoculations, none of the reduced vaccine schedules as tried in our present and previous series (Atanasiu et al., 1956, 1957) can be considered adequate for post-exposure immunization purposes. None of these schedules gave results which compared in efficacy (time of appearance, level and persistence of antibody) with the results from schedules comprising at least 14 daily inoculations of vaccine obtained by ourselves and by other workers (Atanasiu et al., 1956, 1957; Fox et al., 1957; Peck et al., 1955, 1956; Selimov et al., 1959b; Greenberg & Childress, 1960).

Booster inoculations

In individuals who have been effectively sensitized previously (see also " Possible pre-exposure immunization schedules " below) the effectiveness of a booster dose of vaccine has been confirmed with the demonstration that a rise in serum antibody appears between four and eight days after the booster. Our own earlier studies (Atanasiu et al., 1957) and the results of other workers (Fox et al., 1957; Selimov et al., 1959b; Anderson et al., 1960; Greenberg & Childress, 1960; Tierkel et al.[1]) have shown that a single booster inoculation of vaccine given intradermally or subcutaneously to individuals who had received courses of antirabies vaccine four months to over 20 years previously resulted in circulating antibody. While this did not invariably occur in all individuals, a rise in antibody was almost always found in those who had at one time shown detectable antibody. For this reason the WHO Expert Committee on Rabies (1960) recommended that a single booster dose of vaccine be given in cases of mild exposure of individuals who have demonstrated an antibody response to antirabies vaccination received in the past. For severe exposure, because of the

[1] Tierkel, E. S. et al. (1961) Unpublished working document WHO/Rabies/144 and unpublished observations.

greater risk involved, the Committee recommended the administration of antirabies serum and a full course of vaccine.

Interference of immune serum with antigenic action of vaccine

The interference of immune serum with the antigenicity of subsequently administered vaccine noted previously (Atanasiu et al., 1957) was confirmed once again. This interference, however, could be overcome by the administration of a sufficient amount of vaccine, as seen in our previous work (Atanasiu et al., 1957) and in that of others (Selimov et al., 1959b). Our present trials indicated that HEP was superior to the duck embryo and phenolized vaccines in this respect. In our present results this interference by serum given at the start of a schedule occurred not only with the active antibody response to the primary course of vaccine, but also with the response obtained after a booster dose. In addition, the use of immune serum in conjunction with a booster dose of vaccine interfered with the booster effect.

Since immune serum probably plays a paramount role as a life-saving factor in severe human exposure to rabies (Baltazard & Bahmanyar, 1955 ; Habel & Koprowski, 1955; Selimov et al., 1959a), the issue to be met is how to minimize this interfering effect. Previous work had indicated that a delay of 24 hours in administering immune serum following inoculation of the first dose of vaccine may lessen the interfering effect (Fox et al., 1957), and this appeared to be confirmed in the present trials. However, such a delay in a treatment procedure would not appear to be indicated in view of observations showing that early administration of serum increases the chances of protection (Habel, 1954a; Habel & Koprowski, 1955; Lépine; [1] Veeraraghavan & Subrahmanyan, 1960; Schindler [2]). Therefore, where serum is indicated it should be administered as soon as possible along with a minimum of 14 daily doses of vaccine, as recommended by the WHO Expert Committee on Rabies (1960). The Expert Committee also recommended that, in order to overcome the interference of serum, when such serum was given with vaccine, one or two supplemental doses of vaccine, of nonnervous tissue origin if possible, should be given in addition 10 and 20 days after the last usual dose. The Committee recognized, however, that the optimum schedules of the combination of serum and vaccine for immunization, including boosters, require further study.

Possible pre-exposure immunization schedules

Although the elaboration of adequate pre-exposure immunization schedules would have relatively limited application in terms of the number of people involved, studies in this field have given valuable information relevant to the immunology of rabies. The schedules tried in our present series were based on our previous work (Atanasiu et al., 1956, 1957) and on that of Fox et al. (1957).

In our present trials the HEP vaccine was given intradermally, and the duck embryo and phenolized vaccines were given subcutaneously. In the schedules used, the HEP vaccine, when inoculated intradermally, tended to give superior results to those obtained with the duck embryo and phenolized vaccines.

Anderson et al. (1960) administered duck embryo vaccine intradermally (0.2 ml per dose). Three inoculations were given to 49 individuals at 5-day intervals, followed by a booster inoculation about 6 months later; 75.5% responded with antibody to the three primary inoculations, and 95.6% responded to the booster dose. Tierkel et al. (*op. cit.*) inoculated volunteers intradermally with HEP and duck embryo vaccine in a dose of 0.2 ml. Three inoculations one week apart were given, followed by a booster six weeks after the third inoculation. Positive antibody responses to the entire series, including the booster dose, were observed in 15 of 42 (36%) and 28 of 56 (50%) in two groups receiving HEP; and in 20 of 42 (48%) and 49 of 61 (80%) in two groups receiving duck embryo vaccine.

Although a comparison of results obtained from different batches of vaccine has limitations, it may be concluded from the above results that any potent vaccine can be used for pre-exposure immunization in persons at high risk. For such immunization the element of time—i.e., early appearance of antibody—is not essential. Adequate spacing of inoculations seems to be as important as the total dosage of antigen given. A reasonable and convenient schedule would be three or four doses given seven days apart, followed by a booster dose between one and two months after the first inoculation.

It will be noted that *none* of the reduced schedules of immunization intended for pre-exposure purposes

[1] Lépine, P. et al. (1956) Unpublished working document WHO/Rabies/85.

[2] See the note by R. Schindler on page 127 of this issue.

has resulted in an antibody response in *all* the vaccinated individuals. Therefore, as recommended by the WHO Expert Committee on Rabies (1960), if pre-exposure immunization is carried out with any vaccine, it is desirable that a detectable antibody response should be tested for on a serum sample obtained after the completion of vaccination. Booster doses should be repeated until antibody is detectable.

* * *

Since the foregoing was written an article has appeared on HEP trials in man similar to those discussed in this paper (Ruegsegger et al., 1961).

RÉSUMÉ

Depuis plusieurs années, les auteurs cherchent à mettre au point un schéma de vaccination contre la rage, qui permettrait de réduire le nombre des injections normalement requises pour une protection efficace, ainsi que la quantité de tissu nerveux hétérologue inoculée.

Les essais, dont les premiers résultats ont été publiés dans le Bulletin en 1956 et 1957, portaient sur l'injection combinée de vaccin et de sérum à haute teneur en anticorps (sérum hyperimmun). Plusieurs types de vaccins ont été employés; vaccin phéniqué (Semple), vaccin préparé sur embryon de canard, vaccin préparé par un nombre élevé de passages (HEP) sur embryon de poulet. Leur efficacité a été déterminée par l'épreuve de neutralisation sur la souris. Cet article rend compte d'une troisième série d'essais, dont les résultats sont les suivants:

Les schémas de vaccination comportant un nombre réduit d'injections (2-7, à divers intervalles de temps) n'ont pas donné de preuves d'efficacité (évaluées d'après le moment d'apparition, le niveau et la persistance des anticorps) comparables à celles que l'on obtient avec 14 injections quotidiennes de vaccin. En conséquence, aucun de ces schémas réduits ne permet de protéger à coup sûr une personne mordue, n'ayant pas été vaccinée antérieurement.

Chez les sujets préalablement sensibilisés, l'efficacité de l'injection de rappel a été confirmée: une élévation du niveau des anticorps apparaît 4-8 jours après l'injection de rappel.

L'efficacité de la sensibilisation paraît dépendre aussi bien de l'intervalle entre les injections que de la quantité totale d'antigène injectée. Un intervalle de 15-20 jours semble être la durée minimum requise pour assurer une réponse-anticorps optimum.

L'action empêchante de l'immunsérum sur la production active d'anticorps par le vaccin a été confirmée à nouveau. Elle peut cependant être éliminée par l'emploi d'une quantité assez élevée de vaccin. Le vaccin HEP sur embryon de poulet semble plus apte que les autres types de vaccin à dominer cette action empêchante du sérum. Cette dernière ne se manifeste pas seulement lors de la première injection de vaccin, mais même lors de l'injection de doses de rappel. L'emploi de sérum lors d'une injection de rappel nuit à la production de l'effet de rappel.

Un intervalle de 24 heures entre la première administration de vaccin et celle de l'immunsérum diminue l'action empêchante, mais, pratiquement, il n'est pas recommandé d'appliquer ce délai, car le retard dans l'administration du sérum diminue les chances de protection.

Diverses conséquences pratiques de ces études peuvent être mentionnées:

Si l'on administre du sérum et du vaccin, après morsure, il faut procéder à 14 injections quotidiennes de vaccin, au minimum; 10-20 jours plus tard, on donnera une ou deux doses supplémentaires de vaccin, préparé sur tissu non nerveux, dans la mesure du possible.

Pour l'immunisation prophylactique des personnes particulièrement exposées aux risques de morsures, n'importe quel vaccin actif peut être utilisé. Une posologie appropriée comprend 3 ou 4 doses de vaccin, à 7 jours d'intervalle, et une dose de rappel 1-2 mois après la première injection de vaccin. Il y a lieu de vérifier la réponse-anticorps sur un échantillon de sérum, après que le schéma complet de vaccination a été effectué. Il faut continuer d'administrer des doses de rappel, jusqu'à ce que des anticorps soient décelables.

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