Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1956, 14, 593-611

RABIES NEUTRALIZING ANTIBODY RESPONSE TO DIFFERENT SCHEDULES OF SERUM AND VACCINE INOCULATIONS IN NON-EXPOSED PERSONS *

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Manuscript received in December 1955

SYNOPSIS

Groups of adult humans, previously unexposed to rabies and with no history of rabies vaccination, were inoculated with different schedules of phenolized inactivated vaccine and Flury strain chicken-embryo vaccine, with or without one inoculation of hyperimmune serum. Serum specimens of the inoculated individuals were studied for antibody up to the 28th day following the first inoculation of the vaccines and serum. The results can be summarized as follows:

1. Passive antibody appeared in the blood-stream within one day following inoculation of hyperimmune serum. The antibody persisted at a good level for at least 10 days, but dropped slightly by the 14th day and was present in most individuals at the 21st day.

2. There was a tendency for the antibody levels at 21 and 28 days to be lower in the phenolized vaccine plus antiserum groups than in those groups which received phenolized vaccine alone.

3. Seven or 12 daily inoculations of phenolized vaccine alone produced antibody in most instances by the 10th day and persisted generally through the 28th day.

* This article will also be published, in Spanish, in the *Boletín de la Oficina Sanitaria Panamericana*

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4. Daily inoculations of phenolized vaccine produced a superior antibody response to that derived from the same total amount of vaccine given as a single inoculation.

5. A single inoculation intramuscularly of Flury strain chicken-embryo vaccine of high egg-passage did not produce detectable antibody.

6. The group which received hyperimmune serum followed by 12 daily inoculations of phenolized vaccine showed early and persistent antibody throughout the entire period of test. The antibody levels were comparable to those recently observed in man treated effectively with antiserum-vaccine combination after severe exposure to rabies.[9]

Introduction

The use of biological products for the prevention of rabies in man has consisted principally of a series of inoculations with vaccines prepared from nervous tissues. This procedure, although differing in detail with respect to preparation and composition of the vaccine, is well known to have several serious drawbacks, of which the more prominent are: (1) failure to prevent death, especially following severe exposures where the incubation period is short;[2, 3] (2) paralytic accidents, apparently resulting from a reaction to the nervous tissue in the vaccine;[1, 10, 21] and (3) the necessity to undergo a prolonged and unpleasant course of treatment (usually 14 to 21 daily inoculations).

In an attempt to overcome these drawbacks a series of experiments were undertaken to obtain information on the possibilities of increasing the efficiency of protection and of reducing the number of inoculations and amount of nervous tissue involved.

Evaluation of any proposed modifications of standard schedules of rabies prophylaxis under field conditions is impracticable because of the large number of exposed persons requiring treatment before statistically significant numbers of cases can be accumulated. Furthermore, there is great reluctance to try any new procedure for the prevention of a disease as uniformly fatal as rabies, and obviously it is impossible to include an untreated control group in any such study. Therefore, the quantitative determinations of neutralizing antibody in the blood in response to passive and active immunization of human beings was selected as the most logical indirect approach to this problem, despite certain limitations of the tests involved and incomplete knowledge of the significance of neutralizing antibody in preventing rabies in man (see " Discussion " on page 601). Some of the preliminary results of this work were summarized in the second report of the Expert Committee on Rabies.[30] The detail and subsequent observations are given in the present paper.

Materials and Methods

Inoculation and bleeding schedules

Adult human volunteers with no previous history of exposure to rabies or of preventive inoculations for the disease were divided into 11 groups of 10 individuals each, and were inoculated as follows:

Group A: Hyperimmune serum (0.5 ml per kg of body-weight) plus 7 daily inoculations of phenolized vaccine (0.5 ml of 20% tissue suspension). The first inoculation of vaccine was started 24 hours after serum inoculation.

Group B: Seven daily inoculations of phenolized vaccine.

Group C: Hyperimmune serum plus 7 daily inoculations of phenolized vaccine. The first inoculation of vaccine was started 7 days after inoculation of the serum.

Group D: Hyperimmune serum plus one large dose of phenolized vaccine (3.5 ml of 20% tissue suspension) given 7 days after inoculation of the serum.

Group E: One large dose of phenolized vaccine (3.5 ml of 20% tissue suspension).

Group F: Hyperimmune serum plus 3 ml Flury vaccine (70% tissue concentration) given 24 hours after the serum.

Group G: One inoculation of 3 ml Flury vaccine.

Group H: Hyperimmune serum plus 3 ml Flury vaccine given 7 days after serum inoculation.

Group I: Hyperimmune serum alone.

Group J: Hyperimmune serum plus 12 daily inoculations of phenolized vaccine (0.5 ml of 20% tissue concentration). The first inoculation of vaccine was given 24 hours after inoculation of the serum.

Group K: Twelve daily inoculations of phenolized vaccine.

Note. There was an insufficient quantity of vaccine to make 14 inoculations for Groups J and K, as originally planned.

Inoculations of the phenolized vaccine were made subcutaneously in a linear arrangement on the abdominal wall as is usually done in rabies vaccine inoculations. The hyperimmune serum and Flury strain vaccine were inoculated intramuscularly.

Venous blood, in 10-ml to 20-ml quantities, was withdrawn into sterile vacuum venules as follows: [a]

Groups A and F: days 0, 1, 3, 7, 10, 14, 21, 28
Groups C, D, H, and K: days 0, 3, 7, 10, 14, 21, 28
Groups B, E, G, and J: days 0, 7, 10, 14, 21, 28
Group I: days 0, 1, 3, 7, 10, 14.

[a] Day 0 = pre-inoculation specimen; day 1 = specimen drawn 1 day after inoculation; etc.

Serum specimens

The blood was allowed to clot, and the serum was separated usually on the same day. Aliquots of the serum were divided into several all-glass ampoules which were then flame-sealed and held at 4°C. The serum specimens were shipped by air in refrigerated cases to the various laboratories and in most instances were received in a cool state within 36 hours following shipment.

Vaccine

The phenolized vaccine was a 20% suspension of goat brain infected with fixed rabies virus, inactivated with phenol;[a] 0.5 ml per inoculation was given, except for groups D and E (see above). A Habel potency test on the vaccine,[8] performed at the time of its preparation (approximately 5 months before its use in these experiments) showed an LD_{50} protection value of 70 470. Unfortunately, no vaccine was left for potency testing following the completion of the series of inoculations.

The Flury vaccine was a 70% suspension of whole chick-embryo infected with the high egg-passage Flury strain of rabies virus.[12, b] The preparation was kept freeze-dried and reconstituted in saline just before injection. The vaccine had satisfactorily passed the guinea-pig potency tests used for this type of vaccine.[13, 20]

Hyperimmune serum

This was a refined hyperimmune horse serum,[b] of which 0.5 ml per kg of body-weight was administered intramuscularly. The level of neutralizing antibody in the serum was determined immediately before and after its use in these experiments. No significant difference was revealed. Serum dilutions of between 1:1000 and 1:2300 neutralized 400 to 1200 LD_{50} of virus in the neutralization test in mice (see below). This potency is approximately equal to the potency of the International Standard Antirabies Serum.[20]

Serum-virus neutralization test

The serum specimens of the inoculated individuals were divided among 8 different laboratories, so that each laboratory tested all the specimens from at least one individual in each of the groups.[c] The test employed was

[a] The phenolized vaccine was prepared and donated by Pitman-Moore Laboratories, Zionsville, Ind., USA.

[b] The Flury vaccine and hyperimmune serum were prepared and donated by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y., USA.

[c] The eight laboratories were as follows: Department of Tropical Medicine and Public Health, Tulane University, New Orleans, La., USA; Government Virus Laboratory, Haifa, Israel; Lederle Laboratories, Viral and Rickettsial Section, Pearl River, N.Y., USA; National Institutes of Health, Basic Studies Section, Bethesda, Md., USA; National School of Public Health, Madrid, Spain; Institut Pasteur de l'Iran, Teheran; Institut Pasteur, Service des Virus, Paris, France; United States Department of Health, Education, and Welfare, Communicable Disease Center, Virus Laboratory, Montgomery, Ala., USA.

a slight modification of the one described in the WHO monograph, *Laboratory Techniques in Rabies*,[18] as follows:

Preparation and titration of standard fixed virus pool. Each of the participating laboratories was supplied with ampoules of freeze-dried CVS-20, the standard US National Institutes of Health challenge virus, which is a passage strain of the original Pasteur fixed virus. After one intracerebral mouse passage of this virus a large group of mice were inoculated, and on harvest of their brains a standard virus pool was prepared from the supernatant of a 20% suspension of the rabies-infected brains. This pool was ampouled in 1-ml amounts, quick-frozen, and stored at −70°C. At least two ampoules of this frozen pool were thawed rapidly and their contents pooled for each test. For the virus pool to be satisfactory for each test, an intracerebral virus titration in mice had to show an LD_{50} at a dilution no lower than 10^{-5}.

Qualitative and quantitative neutralization tests. Tests for antibody content of the human serum specimens (test sera) were carried out in two stages: first, a qualitative screening test to detect the presence of antibody, and secondly, a quantitative test for antibody content on specimens positive in the screening test.

Neutralization tests on the test sera were arranged so that all serum specimens from one individual were tested at the same time. Controls consisting of negative human serum, and titrations of known positive human serum and the challenge virus, were always carried out in every test. The control sera from one source were supplied to all laboratories.

The test sera in the undiluted state were inactivated for 30 minutes at 56°C in a water-bath. For the qualitative test undiluted sera were mixed with equal volumes of a virus dilution calculated to contain 15-60 LD_{50} of virus, since preliminary tests using 300-3000 LD_{50} failed to reveal antibody in many of the specimens.

All serum-virus mixtures were shaken thoroughly after the virus was added and placed in a 37°C incubator for 1½ hours. Following this they were placed in a pan containing ice-water and held in a refrigerator until inoculated into the mice. (Where a large number of sera were being tested at one time, serum dilutions were made the day before and held in the refrigerator overnight. Virus dilutions were always made the same day mice were inoculated.)

Intracerebral inoculations of 0.03 ml of the serum-virus mixtures were started immediately after the end of the 1½-hour incubation period. Five mice per dilution were inoculated. Control immune serum dilutions were inoculated first, then each of the test sera, and finally the control normal serum containing the challenge virus dilutions (for titration of the virus). Mice were observed daily and all rabies symptoms and deaths recorded. The test was terminated at the end of 14 days. All deaths after the 5th day.

whether preceded by symptoms or not, were considered rabies deaths. Any mice still alive but paralysed at the end of the observation period were also considered rabies deaths.

Positive serum specimens were then selected for quantitative analysis on the basis of their giving the most information for possible future modification of treatment schedules in man. This selection was necessary because of the great requirements in work and material for these tests. Preliminary serum titrations against low LD_{50}'s of virus revealed that low antibody titres were to be expected. A maximum of 3 fivefold dilutions (1:5, 1:25, and 1:125 final dilutions) was therefore considered sufficient to include the end-point of most of the serum specimens, and these dilutions were used in the tests against approximately 30-60 LD_{50} of virus.

Qualitative and quantitative tests were performed 6-12 months and 2 years, respectively, after serum specimens were taken.

The numerical results given in the tables represent the work of one laboratory [a] after preliminary screening tests on the serum specimens had been done in the 8 collaborating laboratories.[b]

Results

Analysis of Table I (Group I): *Hyperimmune serum alone (0.5 ml per kg of body-weight)*

The purpose of Group I was to establish a baseline for antibody levels resulting from the administration of hyperimmune serum alone. Unfortunately, specimens were not taken from this group after the 14th day. However, some indication as to the decline of antibody levels following a dose of antiserum can be obtained from the results in Groups F and H (Tables II and III). In those groups serum was given along with a dose of vaccine which, when used alone (Group G) gave no antibody response. In Group I detectable antibody was present in all serum specimens up to and including the 14th day. It is interesting to note that antibody levels were slightly, although not significantly, higher in most instances on the 3rd day than they were on the first day following inoculation of the serum. The antibody level held well in general until the 10th day, but by the 14th day a decline in serum titre was apparent in 7 out of 10 individuals.

Analysis of Group G: *One inoculation intramuscularly of 3 ml Flury vaccine*

One inoculation of Flury vaccine intramuscularly had no antigenic effect.

[a] Service des Virus, Institut Pasteur, Paris
[b] See footnote c on page 596.

Analysis of Table II (Group F): *Hyperimmune serum plus 3 ml Flury vaccine (70% tissue concentration) given 24 hours after the serum*

Group F indicates that, qualitatively, Flury vaccine does not affect the presence of detectable antibody following the administration of hyperimmune serum. In consideration of the lack of antigenic effect of the Flury vaccine alone shown by Group G, the results of Group F can be interpreted as representing for the most part the effect of serum alone, and corroborate and extend, from a qualitative viewpoint, the results listed in Table I (Group I). Passive antibody persisted in most of the individuals at the 21st day but 3 out of 10 individuals had no detectable antibody by the 28th day.

Analysis of Table III (Group H): *Hyperimmune serum plus 3 ml Flury vaccine given 7 days after serum inoculation*

These results corroborate those of Table II (Group F), and the same general comments hold.

Analysis of Table IV (Group E): *One large dose of phenolized vaccine (3.5 ml of 20% tissue suspension)*

One large dose of phenolized vaccine, equal in total quantity to that administered in Group B (Table VI), provided detectable antibody in only 4 out of 10 individuals.

Analysis of Table V (Group D): *Hyperimmune serum plus one large dose of phenolized vaccine (3.5 ml of 20% tissue suspension) given 7 days after inoculation of the serum*

The effect of hyperimmune serum was again apparent here (see also Tables I, II, and III). Passive antibody was still present at the 21st day and perhaps persisted in some individuals to the 28th day, if the effect of vaccine alone as given in Table IV was approximately the same.

Analysis of Table VI (Group B): *7 daily inoculations of phenolized vaccine*

Compare with Table IV, Group E. The administration of the same quantity of antigen in 7 daily inoculations instead of a single inoculation

exerted a markedly superior antigenic effect. Antibody began to appear in most instances by the 10th day, the titre increased by the 14th day through the 21st day and persisted in 9 out of 10 individuals through the 28th day.

Analysis of Table VII (Group A): *Hyperimmune serum plus 7 daily inoculations of phenolized vaccine. First inoculation of vaccine started 24 hours after serum inoculation.*

Compare with Table VI, Group B. The effect of serum is seen here in the uniformly positive specimens before the 10th day. Subsequent antibody levels through the 28th day are inferior, on the whole, to those encountered in Group B, which received the same course of vaccine without serum.

Analysis of Table VIII (Group C): *Hyperimmune serum plus 7 daily inoculations of phenolized vaccine. First inoculation of vaccine started 7 days after inoculation of the serum.*

This group corroborates qualitatively the results for Group A in Table VII. Administration of the vaccine was delayed until the 7th day after serum inoculation, in case a marked interfering effect of serum and vaccine should be encountered in Group A. Note that no antibody was detected on the 14th day specimens of C3 and C9, followed by the reappearance of antibody.

Analysis of Table IX (Group K): *12 daily inoculations of phenolized vaccine*

With 12 daily inoculations of vaccine, antibody began to appear at the 10th day (see also Table VI, Group B), increased slightly, and persisted through the 28th day. Quantitatively, the results do not appear to be superior to those in Table VI (Group B), where only 7 inoculations were used.

Analysis of Table XI (Group J): *Hyperimmune serum plus 12 daily inoculations of phenolized vaccine. First inoculation of vaccine given 24 hours after inoculation of the serum.*

The addition of serum to the 12 inoculations of phenolized vaccine (compare with Table IX, Group K) resulted in early antibody in all individuals; antibody levels fluctuated slightly from the 10th through the 21st day, but a definite decline was observed in 5 out of 10 individuals by the 28th day.

Discussion

Limitations of experimental arrangement

The reproducibility of results and the significance to be given to the numerical end-points of the quantitative tests are obviously important considerations in any study designed to compare the efficacy of different courses of immunization based on antibody response.

During the course of this study there was an opportunity to check on the results obtained in the qualitative tests carried out in the eight participating laboratories, since most originally positive sera were subsequently retested either qualitatively or quantitatively in a single laboratory and again found to be positive. However, it became apparent that serum dilution end-points in quantitative tests could vary depending on the number of LD_{50}'s of virus used in the test. The amount of virus mixed with the serum specimens was also found to influence the results in the qualitative tests when borderline amounts of antibody were involved. As little as a fivefold increase in the number of LD_{50}'s used could make a positive serum become negative or reduce the neutralization end-point in a quantitative test.

An attempt was made to keep this important variable at a minimum in this study. Qualitative tests showing negative results with a large amount of virus were rechecked in tests with less virus; quantitative tests were run in one laboratory and against 30-60 LD_{50} of virus with all sera from any one individual and individuals from strictly comparable groups included in a single test on any one day; standard positive and negative control sera were quantitated in every test. However, in spite of these precautions it is important to emphasize that the numerical end-point of the serum neutralization titre as recorded in the tables must not be taken too strictly in comparing results in individuals or groups, since slight variation in the amount of virus from test to test may have caused some variation in the results. This quite probably is the explanation for the fact that in several individuals the level of antibody seemed to fluctuate from one level at one bleeding to a lower level in the next sample, and to rise again subsequently.

Nevertheless, in spite of these inherent biological limitations to the quantitative aspects of the test results, there is an obvious consistency of general level of antibody between serial specimens in individuals, between individuals in each group, and between groups receiving similar treatment. For this reason it is felt that comparisons of the efficacy of the different treatment schedules are valid on the following basis:

(1) time of appearance and disappearance of antibodies;

(2) general quantitative level of antibody at any one period in the course of treatment;

(3) trend of antibody levels up or down at certain time intervals.

Although the tests were performed in batches over a period of almost two years, there was no evidence of a change in antibody levels due to storage of the sera.

Relationship and significance of neutralizing antibody to protection against rabies infection

It is difficult to evaluate conflicting reports based on work up to a few years ago concerning the correlation of neutralizing antibody to protection in man and animals.[4, 7, 11, 19, 22, 25, 28] Much of this difficulty lies in the varied procedures used for detecting antibody, and the consequent incomparability of the results obtained. In the past few years, however, more uniform techniques have been used and this recent work will be cited.

In cattle there seems to be a good correlation between a medium to high antibody level and resistance to challenge infection with street virus; however, the correlation appears to be less consistent when antibody levels are quite low.[9] In dogs, while the presence of antibody usually indicates resistance, absence of antibody does not necessarily signify susceptibility.[14, 26, 27] In guinea-pigs there is a direct correlation between antibody levels, even of low titre, and resistance to challenge with street virus,[15] and this was found to be generally true in mice also.[17, a]

Only recently has there become available any information on this question with respect to man. Habel & Koprowski [9] analysed serum specimens of 17 individuals severely bitten by a single rabid wolf. These individuals were divided into three groups which received, respectively one inoculation of serum plus a course of vaccine, two inoculations o serum five days apart and a course of vaccine, and a course of vaccine alone. Apart from a single blood specimen showing a 50% end-point at a serum dilution of 1:191, no titre exceeded 1:112; in fact, only a very few were higher than 1:50. Eight to 46 mouse LD_{50} were used in the serum-neutralization test, which was carried out according to the technique and using the materials given earlier in this paper in the section " Materials and Methods " (see page 595). Although the authors could not draw conclusions correlating the level of antibody with protection of any exposed individual, it was possible for them to state that

" . . . It is apparent that antibody demonstrable early 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."

Thus, if we take the presence of early and continued antibody levels as an indicator of protection against rabies exposure in man, only Group J (serum plus 12 daily inoculations of phenolized vaccine) fulfilled this requirement completely in the groups tested, although nothing can be said

[a] See also the article by K. Habel on page 613 of this number of the *Bulletin*.

concerning the level of antibody beyond the 28-day period of observation. Group I was also satisfactory up to the 14th day, when the last serum specimen was taken for the group, but the results obtained with Groups F and H indicate that antibody obtained from the single dose of serum alone is not consistently retained beyond the 21st day. Although, as stated previously, there is insufficient information to make inferences concerning quantitative levels of antibody necessary to afford protection against severe exposure to rabies, it may be of interest to note that in the wolf-bite series already referred to the serum specimens of both survivors and eventual fatalities generally showed lower antibody levels than Group J. This indicates that the dosages of serum and vaccine used in the present experiment would perhaps be adequate in severe field exposure.

The question arises of possible interference between the antigen, in the form of vaccine, and antibody in the antiserum. There was no evidence that phenolized vaccine antigen reduced the early antibody levels produced by the antiserum. With respect to the early presence of antibody from the antiserum possibly affecting the later antibody response caused by the phenolized vaccine, the data in these experiments are inconclusive. There appears to be a tendency for the antibody levels at 21 and 28 days to be lower in the vaccine plus antiserum groups (A and J) than in the similar groups (B and K) receiving vaccine alone. However, if a partial interference of antiserum with antigen action by phenolized vaccine did in fact occur, the paramount role played by antiserum as a life-saving factor in severe human exposures to rabies [3, 9] outweighs any consideration which may be given to the possible interference.

With respect to possible interaction of Flury vaccine and antiserum, no effect was discernible in the present experiments. Other workers have observed no interference between Flury vaccine and antiserum from the standpoint of effective protection conferred in guinea-pigs and dogs subjected to challenge with street virus.[16, a]

Although no serum specimens in the present experiments were taken beyond the 28th day, long persistence of antibody has been demonstrated by LeBell and his co-workers [20] in an investigation on persons comparable to Groups B and K in our series. They tested serum specimens of 18 individuals who received 7 daily inoculations of phenolized vaccine as in Group B, and of 69 individuals who received 14 daily inoculations of the vaccine (as compared with 12 inoculations in Group K). Detectable antibody was present in most individuals of both groups 8-12 months following the first inoculation of the vaccine.

[a] It should be noted that although there was a failure in the present experiments to produce antibody response in man with one intramuscular injection of Flury strain vaccine, success has been achieved elsewhere when two or more intramuscular or intradermal inoculations were used at spaced intervals [5, 24].

TABLES I-X. EXPLANATORY NOTE

Quantitative determinations are given as whole numbers representing the serum dilution (calculated according to the method of Reed & Muench [23]) which protected 50 % of the inoculated mice. Three fivefold serum dilutions (final 1 : 5, 1 : 25, and 1 : 125) were used in these tests after preliminary screening tests on the serum specimens had been done using a 1 : 2 final dilution of serum. Thus, <5 indicates that the end-point lies between 1 : 2, the final dilution used for the screening test, and 1 : 5, the lowest dilution used for subsequent quantitative analysis; >125 indicates that the protective end-point was not reached with the final 1 : 125 dilution of serum.

All quantitative tests recorded in the tables were carried out with 30-60 LD_{50} of virus.

Qualitative tests are recorded as + (positive), — (negative), or ± (doubtful or trace). Positive indicates survival of at least 4 out of 5 mice used for each serum specimen; the survival of 2 or 3 of the 5 mice is interpreted as doubtful or trace, and survivals below 2 are interpreted as negative.

Pre-inoculation serum specimens were examined in all instances and were negative. These data are not included in the tables.

TABLE I. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP I *

Hyperimmune serum alone (0.5 ml per kg of body-weight)

Individual	Days following inoculation				
	1	3	7	10	14
I1	<5	14	+	11	<5
I2	9	55	+	6	+
I3	19	20	+	<5	<5
I4	8	10	+	14	<5
I5	14	55	+	17	6
I6	9	13	+	25	11
I7	19	11	+	11	42
I8	9	19	+	6	7
I9	7	11	+	11	<5
I10	14	9	+	25	25

* See *Explanatory Note* above.

GROUP G : ONE INOCULATION OF 3 ML FLURY VACCINE

In Group G, who received one intramuscular injection of 3 ml of Flury vaccine, negative results were obtained in all 10 individuals with serum specimens drawn 7, 10, 14, 21, and 28 days following inoculation.

TABLE II. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP F *

Hyperimmune serum (0.5 ml per kg of body-weight) plus 3 ml Flury vaccine (70 % tissue concentration) given 24 hours after serum

Individual	Days following first inoculation						
	1	3	7	10	14	21	28
F1	+	+	+	+	+	NT	NT
F2	+	+	+	+	+	+	+
F3	+	+	+	+	+	±	—
F4	+	+	+	+	+	+	—
F5	+	+	+	+	+	+	+
F6	+	+	+	±	+	—	—
F7	+	+	+	+	+	+	+
F8	+	+	+	+	+	+	+
F9	+	+	+	+	+	+	±
F10	+	+	+	+	+	+	±

* See *Explanatory Note* on opposite page. NT = not tested (insufficient serum)

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I2	9	55	+	6	+
I3	19	20	+	<5	<5
I4	8	10	+	14	<5
I5	14	55	+	17	6
I6	9	13	+	25	11
I7	19	11	+	11	42
I8	9	19	+	6	7
I9	7	11	+	11	<5
I10	14	9	+	25	25

* See *Explanatory Note* above.

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Individual	Days following first inoculation						
	1	3	7	10	14	21	28
F1	+	+	+	+	+	NT	NT
F2	+	+	+	+	+	+	+
F3	+	+	+	+	+	±	—
F4	+	+	+	+	+	+	—
F5	+	+	+	+	+	+	+
F6	+	+	+	±	+	—	—
F7	+	+	+	+	+	+	+
F8	+	+	+	+	+	+	+
F9	+	+	+	+	+	+	±
F10	+	+	+	+	+	+	±

* See *Explanatory Note* on opposite page. NT = not tested (insufficient serum)

TABLE III. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP H *

Hyperimmune serum (0.5 ml per kg of body-weight) plus 3 ml Flury vaccine given 7 days after serum inoculation

Individual	Days following first inoculation					
	1	7	10	14	21	28
H1	+	+	+	+	+	+
H2	+	+	+	+	+	−
H3	+	+	+	+	+	−
H4	+	+	+	+	±	±
H5	+	+	+	+	+	+
H6	+	+	+	+	+	+
H7	+	+	+	+	+	−
H8	±	±	±	±	±	±
H9	+	+	+	+	+	±
H10	+	±	−	−	−	−

* See *Explanatory Note* on page 604.

TABLE IV. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP E *

One large dose of phenolized vaccine (3.5 ml of 20 % tissue suspension)

Individual	Days following inoculation				
	7	10	14	21	28
E1	−	−	−	−	−
E2	−	−	+	+	+
E3	−	−	−	−	+
E4	±	+	±	+	+
E5	−	−	−	−	NT
E6	−	−	−	−	−
E7	−	−	−	−	−
E8	−	−	−	−	−
E9	−	±	±	−	−
E10	−	+	+	+	+

* See *Explanatory Note* on page 604.

NT = not tested (insufficient serum)

TABLE V. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP D *

Hyperimmune serum (0.5 ml per kg of body-weight) plus one large dose of phenolized vaccine (3.5 ml of 20 % tissue suspension) given 7 days after inoculation of serum

Individual	Days following first inoculation					
	1	7	10	14	21	28
D1	+	+	+	+	+	+
D2	+	+	+	+	+	−
D3	+	+	+	−	−	−
D4	+	+	+	+	+	+
D5	±	+	+	+	+	+
D6	+	+	+	+	+	+
D7	+	+	+	+	+	+
D8	+	+	+	+	−	−
D9	+	+	+	+	+	+
D10	+	+	+	+	+	±

* See *Explanatory Note* on page 604.

TABLE VI. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP B *

Seven daily inoculations of phenolized vaccine (0.5 ml of 20 % tissue suspension)

Individual	Days following first inoculation				
	7	10	14	21	28
B1	−	−	25	15	7
B2	+	9	25	93	>125
B3	−	−	<5	36	32
B4	−	<5	15	25	11
B5	−	<5	<5	<5	−
B6	−	<5	14	17	<5
B7	−	37	125	>125	>125
B8	−	<5	14	21	46
B9	−	<5	7	55	76
B10	−	<5	6	12	<5

* See *Explanatory Note* on page 604.

TABLE VII. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP A *

Hyperimmune serum (0.5 ml per kg of body-weight) plus 7 daily inoculations of phenolized vaccine (0.5 ml of 20 % tissue suspension). First inoculation of vaccine started 24 hours after serum inoculation.

Individual	Days following first inoculation						
	1	3	7	10	14	21	28
A1	11	12	+	<5	10	<5	—
A2	17	15	+	11	<5	<5	—
A3	11	17	+	<5	<5	—	—
A4	<5	19	+	15	9	8	<5
A5	11	10	+	<5	13	<5	<5
A6	8	25	+	25	11	12	<5
A7	11	14	+	11	10	<5	<5
A8	9	14	+	13	8	11	<5
A9	17	49	+	11	<5	<5	<5
A10	<5	30	+	11	19	<5	<5

* See *Explanatory Note* on page 604.

TABLE VIII. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP C *

Hyperimmune serum (0.5 ml per kg of body-weight) plus 7 daily inoculations of phenolized vaccine (0.5 ml of 20 % tissue suspension). First inoculation of vaccine started 7 days after serum inoculation.

Individual	Days following first inoculation					
	1	7	10	14	21	28
C1	+	+	+	+	+	±
C2	+	+	+	+	+	+
C3	+	+	+	—	+	+
C4	+	+	+	+	+	+
C5	+	+	+	+	+	+
C6	+	+	+	+	—	—
C7	+	+	+	+	+	+
C8	+	+	+	+	—	—
C9	+	+	+	—	+	+
C10	+	+	+	+	+	+

* See *Explanatory Note* on page 604.

TABLE IX. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP K *

Twelve daily inoculations of phenolized vaccine (0.5 ml of 20 % tissue suspension)

Individual	Days following first inoculation					
	1	7	10	14	21	28
K1	—	—	—	+	<5	<5
K2	—	—	+	>125	>125	69
K3	—	+	+	93	>125	93
K4	—	—	—	—	<5	<5
K5	—	—	—	+	+	7
K6	—	—	<5	+	25	NT
K7	—	—	<5	32	+	>125
K8	—	—	<5	17	+	32
K9	—	—	<5	±	42	+
K10	—	—	<5	55	125	>125

* See *Explanatory Note* on page 604. NT = not tested (insufficient serum)

TABLE X. VIRUS NEUTRALIZING ANTIBODIES IN SERUM SPECIMENS TESTED WITH 30-60 LD_{50} OF VIRUS — GROUP J *

Hyperimmune serum (0.5 ml per kg of body-weight) plus 12 daily inoculations of phenolized vaccine (0.5 ml of 20 % tissue suspension). First inoculation of vaccine started 24 hours after serum inoculation.

Individual	Days following first inoculation				
	7	10	14	21	28
J1	+	13	12	89	<5
J2	+	NT	89	<5	<5
J3	+	15	80	25	25
J4	+	125	5	>125	5
J5	+	89	11	87	75
J6	+	<5	<5	>125	>125
J7	+	32	19	25	89
J8	+	11	19	11	<5
J9	+	<5	15	83	<5
J10	+	11	65	65	<5

* See *Explanatory Note* on page 604. NT = not tested (insufficient serum)

RÉSUMÉ

L'inoculation de vaccins préparés sur tissu nerveux, à laquelle on a recouru jusqu'à maintenant pour protéger contre la rage les sujets mordus, présente plusieurs inconvénients: elle n'empêche pas à coup sûr l'infection, surtout dans les cas de morsures graves où la période d'incubation est courte; elle provoque parfois des accidents paralytiques dus au tissu nerveux hétérologue; le traitement, qui s'étend sur une période de 14 à 21 jours, est fastidieux.

Les auteurs ont cherché à mettre au point un schéma de traitement prophylactique qui assurerait une protection efficace tout en réduisant le nombre des injections nécessaires et la quantité de tissu nerveux inoculée. Onze groupes composés chacun de dix sujets adultes n'ayant jamais été mordus par un animal enragé ni vaccinés contre la rage reçurent des injections soit de vaccin phéniqué, soit de vaccin souche Flury cultivé sur embryon de poulet, soit de sérum à haute teneur d'anticorps (sérum hyperimmun), soit de vaccin + sérum, en diverses posologies. Le titrage des anticorps neutralisants dans le sérum des sujets soumis à l'étude a paru être la méthode la plus adéquate d'évaluation de la protection, bien que l'on ne connaisse qu'imparfaitement encore le rôle protecteur de ces anticorps chez l'homme infecté par le virus rabique. Des échantillons de sérum ont été prélevés sur les sujets des divers groupes jusqu'au 28e jour après l'inoculation et soumis aux épreuves de neutralisation dans huit laboratoires. Les résultats peuvent être résumés comme suit:

Des anticorps passifs ont été mis en évidence dans le sang circulant dès le jour qui a suivi l'inoculation de sérum à haute teneur d'anticorps. Ils se sont maintenus à un niveau satisfaisant pendant 10 jours au moins et, malgré un fléchissement à partir du 14e jour, ils ont pu être décelés chez la plupart des sujets jusqu'au 21e jour.

Les sérums des sujets ayant reçu vaccin phéniqué + sérum, examinés les 21e et 28e jours, semblaient moins riches en anticorps que les sérums des groupes n'ayant reçu que le vaccin.

Des inoculations quotidiennes de vaccin phéniqué seul, échelonnées sur 7 ou 12 jours, ont suscité des anticorps titrables dès le 10e jour, qui persistaient encore le 28e jour.

La réponse sérologique à une quantité donnée de vaccin phéniqué a été plus forte lorsque la dose était répartie en plusieurs injections quotidiennes plutôt qu'administrée en dose unique.

Une injection intramusculaire unique de vaccin souche Flury (nombre élevé de passages sur œuf) n'a pas produit d'anticorps décelables.

Seuls les sujets du groupe ayant reçu le sérum à haute teneur d'anticorps puis douze inoculations quotidiennes de vaccin phéniqué ont présenté des anticorps dès le début et jusqu'à la fin de la période d'étude.

Il semble que cette dernière posologie, combinant immunisation passive et immunisation active, permette d'assurer la protection des individus atteints de morsures graves, telle qu'elle a été précisée dans une étude pratique récente.

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