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

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SYNOPSIS

Further studies were made on groups of adult humans, previously unexposed to rabies and with no history of rabies vaccination, who were inoculated with different schedules of phenolized inactivated vaccine given subcutaneously and high egg passage (HEP) Flury strain vaccine given intradermally, with and without inoculation of antirabies serum. Serum specimens of the inoculated individuals were studied for antibody up to the 60th day after the first inoculation of the vaccines and serum. Studies were also made on the effect of "booster" doses of HEP Flury strain vaccine given 6 months after preparatory inoculations. The results 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

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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 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 results of a first series of experiments on antibody response to different schedules of serum and vaccine inoculations in previously non-exposed persons [1] indicated that : (*a*) passive antibody from antirabies serum appeared within one day after inoculation and persisted for at least three weeks; (*b*) serum given at the same time as phenolized vaccine apparently interfered with the antigenic action of the vaccine in the production of antibodies; (*c*) 7 or 12 daily inoculations of phenolized vaccine alone produced antibody in most instances by the 10th day and generally continued to do so through to the 28th day, the limit of the observation period; (*d*) daily inoculation of phenolized vaccine produced a superior antibody response to that derived from the same total amount of vaccine given as a single inoculation; (*e*) a single inoculation intramuscularly of Flury strain chicken-embryo vaccine of high egg passage (HEP) did not produce detectable antibody; and (*f*) persons who received serum followed by 12 daily inoculations of phenolized vaccine showed early and persistent antibody throughout the 28-day period of test.

The present paper reports the results of a second series of experiments designed to clarify and extend the above observations with a view towards increasing the efficiency of protection and reducing the number of inoculations and amount of nervous tissue involved in the use of antirabies vaccines.

Materials and Methods

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

Group L : 10 individuals received 14 daily inoculations of phenolized vaccine A (2 ml of 5% nervous tissue suspension per inoculation).

Group N : 10 individuals received 3 inoculations each of phenolized vaccine A, given 5 days apart.

Group O : Same as group N, plus 1 inoculation of serum (0.5 ml per kg body-weight) given at the same time as the first vaccine inoculation.

Group P : Same as group O, plus a second serum inoculation given on the 5th day (120 hours) after the first serum and vaccine inoculation.

Group Q : 10 individuals received Flury (HEP) vaccine intradermally as follows: 0.2 ml in each arm, followed by 0.2 ml in one arm on 5th day (120 hours) after the first inoculation, and 0.2 ml in the second arm on 10th day after the first inoculation.

Group QQ : 6 individuals from group Q received a " booster " dose of 0.2 ml Flury HEP vaccine in one arm 6 months after the initial inoculation of the vaccine.

Group R : Same as group Q plus 1 serum inoculation given simultaneously with the first vaccine inoculation.

Group RR : 7 individuals from group R received a " booster " dose as in group QQ.

Group QQRR: 4 previously uninoculated individuals received an original dose of 0.2 ml Flury vaccine in one arm to serve as a control group for groups QQ and RR.

Group S : 10 individuals were divided into 2 groups of 5 individuals each ; the first group received one inoculation of serum (0.5 ml per kg body-weight), and the second group received a second inoculation of serum 5 days (120 hours) after the first inoculation.

Group T : 6 individuals received 14 daily inoculations of phenolized vaccine B (0.5 ml of 20% nervous tissue suspension per inoculation).

Group U : Same as group T, plus one inoculation of serum (0.5 ml per kg body-weight) given the same day as the first vaccine inoculation.

Group V : Same as group U, plus a second inoculation of serum given the 5th day (120 hours) after the first inoculation of serum and vaccine.

Venous blood in 20-ml quantities was withdrawn into sterile vacuum venules as follows:

Groups L, N, Q and R: days 0,[a] 10, 15, 21, 28, 60
Groups O, P, T, U and V: days 0, 10, 15, 21, 28, 42, 60

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

Group S: days 0, 2, 6, 10, 15, 21, 28, 42, 60
Groups QQ, RR and QQRR: days 0, 5, 15, 30

Serum specimens

The serum was separated from the clot on the same day of the bleeding and placed in glass ampoules which were flame-sealed and held at either —30°C or + 4°C. The specimens were shipped by air in refrigerated cases to the various laboratories and were received in a cool state within 36 hours of shipment. Serum specimens were examined for antibody content within nine months after they had been obtained from the inoculated individuals.

Vaccine

Two different batches of phenolized vaccine were used for the inoculations because some of the groups were inoculated six months apart, and experience has shown that phenolized vaccines do not remain stable for that length of time. Vaccine A was inoculated into groups L, N, O, P—each group within a few days of the other— and vaccine B was likewise inoculated into groups T, U, V.

Vaccine A[a] was 5% goat brain suspension of phenolized vaccine which showed slightly more than 1000 LD_{50} protection in the Habel mouse test for potency[8] one month before inoculation of the individuals. A dose of 2 ml per inoculation was used.

Vaccine B[b] was a 20% rabbit brain suspension which showed slightly more than 3000 LD_{50} protection at the time of inoculation. A dose of 0.5 ml per inoculation was used, thereby giving a total amount of nervous tissue equivalent to vaccine A.

Inoculations of the phenolized vaccines were made subcutaneously in a linear arrangement on the abdominal wall, as is usually done in rabies vaccine inoculations.

The Flury HEP vaccine was a 35% suspension of whole chicken-embryo infected with the 197th egg passage of the Flury strain of virus.[5] The preparation was freeze-dried and reconstituted in saline just before intradermal injection (0.2 ml) on the inner surface of the forearm. The vaccine had satisfactorily passed the guinea-pig potency tests used for this type of vaccine[9, 10] and showed no drop in potency when retested by a modified potency test in mice after the human inoculations had been made. This modified test consisted of intracerebral inoculations of tenfold dilutions of the vaccine (10^{-2} to 10^{-6}), followed by intracerebral challenge with street virus; a $10^{-6.25}$ dilution of vaccine protected against approximately 5600 LD_{50} of street virus, which was only slightly inferior to a similar potency test made at the time of production of the vaccine.

[a] Kindly supplied by Pitman-Moore Division, Allied Laboratories, Indianapolis, USA
[b] Kindly supplied by Merck Sharpe and Dohme, Philadelphia, USA

Antirabies serum

This was a refined serum from hyperimmunized horses;[a] 0.5 ml per kg body-weight was inoculated intramuscularly at different sites in the buttocks and thigh. A potency test of this serum after the human inoculations had been made showed a protective titre of 1:5164 against 239 LD_{50} of fixed virus. This result met the requirements of therapeutic serum in the mouse potency test recommended in the second report of the WHO Expert Committee on Rabies.[9] A slightly modified potency test has since been recommended in the third report of the Committee.[10]

Serum neutralization (SN) tests

"Regular" SN test. This test procedure was given in detail in our previous report[1] and will be referred to in this paper for convenience as the "regular" SN test. It consisted first of screening serum specimens for detectable antibody at a 1:2 final dilution of serum against 8-100 LD_{50} of fixed virus; after incubation at 37°C for one and a half hours the mixture was inoculated intracerebrally into mice. All positive specimens were then re-tested for quantitative purposes, using final serum dilutions of 1:5, 1:25 and 1:125 (see Tables I-VII).

When all the SN tests ("regular" and "modified") had been completed it appeared likely that some sera which may have been positive were being missed (individuals P2 and P5 in Table IV, individuals U1-U4 in Table VI, individual V4 in Table VII). It was therefore decided to re-test some of these specimens by the following method: instead of using a 1:2 final dilution of serum as in the "regular" test, we adjusted the quantity of virus suspension so that 1 part of virus was added to 19 parts of serum giving a virtually undiluted serum containing 30 LD_{50} of virus. This procedure revealed antibody in 10 out of 12 specimens which had been negative to two previous "regular" SN tests.

"Modified" SN test. A modification of the neutralization test referred to above was performed on some of the serum specimens (Tables VIII-XII) because in comparative tests on the same serum sample the "modified" test showed a slightly greater sensitivity for detecting antibody than did the "regular" test. However, the difference in sensitivity was not great enough to warrant use in all the collaborating laboratories because of the newness of the procedure, and because of some difficulty in obtaining comparable results—a difficulty not experienced with the "regular" test. The use of the "modified" test was therefore confined to two laboratories.

The "modified" test consisted of mixing 9 parts of undiluted serum and 1 part of a suspension of fixed virus containing approximately

[a] Kindly supplied by Lederle Laboratories, American Cyanamid Company, Pearl River, N.Y., USA

10 000 LD_{50}. The mixture was incubated for one and a half hours at 37°C; after incubation, either a 1:100 dilution of the serum-virus mixture was made for the purpose of screening, or fivefold dilutions (1:5 to 1:15 626) were made and inoculated intracerebrally into mice without previous screening. Specific antibody was considered to be present when a difference of more than 1.0 log LD_{50} titre (end-point) was noted between the negative-serum plus virus mixture (control) and the test serum-virus mixture. The screening at a 1:100 dilution of the test serum-virus mixture served as a qualitative test, and specimens which revealed antibody were tested quantitatively with fivefold dilutions as indicated above.

Negative results for all qualitative tests were checked at least twice. Quantitative tests using the "regular" SN test were performed in six different laboratories, each laboratory testing all serum specimens from at least two, and up to five, individuals of a particular group. All questionable results were re-checked in one laboratory. All sera from any one individual, and individuals from strictly comparable groups, were included in a single test on any one day.

Results

Analysis of Table I (Group L): *14 daily inoculations of phenolized vaccine A (2 ml of 5% tissue suspension)*

This group received the usual course of vaccine treatment and served as a basis of reference for other groups receiving different schedules of phenolized vaccine A, with and without serum. The results obtained agreed generally with the previous series [1] but, unlike the latter, where antibody appeared in low levels in 7 out of 10 individuals by the 10th day, detectable antibodies were recorded in the present group in only 1 individual by the 10th day. Eight out of 10 individuals had antibodies by the 15th day, and all showed antibodies by the 21st day. Relatively high levels of antibodies were recorded for 6 out of 10 individuals from the 21st day, which held, with two exceptions, until the 60th day, the last day of the test. Two 60-day specimens were unavailable for testing.

Analysis of Table II (Group N): *3 inoculations of phenolized vaccine A given 5 days apart*

Only 2 individuals showed antibodies by the 10th or 15th day, and 6 by the 21st day. By the 60th day 8 out of 10 showed antibodies. In view of the reduced quantity of antigen used, this can be considered a good response. It should be noted that Fox et al.[2] obtained detectable antibody response in 17 out of 19 individuals by the 15th day, and in all 19 individuals by the 60th day, after 4 inoculations of phenolized vaccine given 5 days apart.

Analysis of Table III (Group O): *Same as Group N (Table II), plus one inoculation of serum (0.5 ml per kg body-weight) given at the same time as the first vaccine inoculation*

Compare with Table II and Table VIII (Groups N and S). All individuals had antibodies on the 10th and 15th days, with a slight falling-off by the 21st and 28th day. Antibodies further declined by the 42nd day, and by the 60th day only 3 out of 10 persons showed antibodies. Table VIII (Group S) shows that passive antibodies persist in some individuals (at least 5 out of 9) until the 42nd day, but they virtually disappear by the 60th day. It is therefore impossible to state whether the antibodies recorded in Table III from the 15th to the 42nd day are passively induced from the serum or actively induced from the vaccine. A comparison of the 60-day specimens of Tables II and III shows clearly that the administration of serum interfered with the antigenic effect of the vaccine when the latter was given in 3 doses 5 days apart.

Analysis of Table IV (Group P): *Same as Group N (Table II), plus two inoculations of serum (0.5 ml per kg body-weight for each inoculation), the serum being given simultaneously with, and 5 days after, the first inoculation of vaccine*

See analysis of Table III. The interference effect of the serum on the antigenic action of the vaccine is again evident, only 1 out of 10 individuals showing antibodies by the 60th day, as compared with 8 out of 10 in Group N (see Table II). It is noteworthy that despite the use of twice the amount of serum as was given to Group O (see Table III), antibody levels, probably of passive origin, on the 10th through to the 21st day were not significantly higher, and were detectable in only 3 (including 1 doubtful) out of 10 individuals by the 42nd day as compared with 8 (including 4 doubtful) out of 10 who received only 1 inoculation of serum. Groups U and V (see Tables VI and VII) also showed that two inoculations of serum did not give antibody levels superior to that given by one inoculation.

It is of interest to note that individuals P2 and P5 were negative to two screening tests with the "regular" SN test, despite their having

received two inoculations of serum; P2 revealed antibodies in the 10- and 15-day specimens, and P5 showed a trace in the 15-day specimens when these sera were tested a third time as described on page 915.

Analysis of Table V (Group T) : *14 daily inoculations of phenolized vaccine B (0.5 ml of 20% tissue concentration)*

This group was set up to serve as a control for Groups U and V (Tables VI and VII) where further evidence was sought of the interference of serum with the antigenic action of vaccine. The results follow in general those obtained with Group L (Table I) where vaccine A was used: 1 out of 6 showed antibodies by the 10th day, 3 by the 15th, and all by the 21st, persisting until the 60th day except for one individual.

Analysis of Table VI (Group U) : *Same as Group T (Table V), plus one inoculation of serum (0.5 ml/kg) given the same day as the first vaccine inoculation*

A third SN test, using what was believed to be a more sensitive technique (see page 915), was required to demonstrate the expected antibodies in the 10- and 15-day specimens. All 6 individuals showed antibodies by the 28th day, with persistence in 5 out of 6 at the 60th day. When this group is compared with Group T (see Table V) there does not seem to be interference on the part of one inoculation of serum with the antigenic action of the vaccine.

Analysis of Table VII (Group V) : *Same as Group T (Table V), plus two inoculations of serum (0.5 ml/kg for each inoculation), the serum being given simultaneously, and 5 days after the first inoculation of vaccine*

Compare with Tables I, V and VI. Passive antibodies from the two inoculations of serum were evident in the 10-day specimens. Antibodies were found in 5 out of 6 individuals until the 28-day specimen, in 4 out of 6 up to the 42nd day, and in only 2 out of 6 on the 60th day. The interference of serum with the vaccine is striking, two serum inoculations exerting a very distinct effect on the antigenic action of the vaccine, in contrast to the group receiving only one serum inoculation.

Analysis of Table VIII (Group S) : *This comprises two groups of 5 individuals each; one group (S1-S5) received 1 serum inoculation (0.5 ml/kg), and the second group (S6-S10) received 2 serum inoculations; the second serum was given 5 days (120 hours) after the first serum inoculation*

One individual (S5) did not show detectable antibody after the second day (except for a doubtful reaction on the 21st day).[a] There appears to be little difference between the groups which received one and two serum inoculations. Passive antibody persisted in 8 (including 1 doubtful) out of 9 individuals tested until the 28th day, but this declined to 5 out of 9 by the 42nd day, and none by the 60th day. Thus, antibodies present in the 60-day specimen of any group were elicited by the vaccine (for example, Group U).

Analysis of Table IX (Group Q) : *Flury HEP vaccine (35% tissue suspension) given intradermally as follows: 0.2 ml in each arm on 0 day; 0.2 ml in one arm on 5th day (120 hours); 0.2 ml in second arm on 10th day*

Doubtful reactions occurred in 4 out of 10 individuals by the 10th day and persisted at a relatively low level until the 28th day. By the 60th day 2 individuals previously negative showed antibodies, in addition to 2 persons definitely positive and 2 with doubtful reactions.

Analysis of Table X (Group R) : *Same as Group Q (Table IX), plus 1 serum inoculation given simultaneously with the first vaccine inoculation*

Antibodies were evident in 7 (including 1 doubtful) out of 9 individuals by the 10th day, and in 2 (including 2 doubtful) out of 9 individuals by the 15th day. They persisted in 7 (including 2 doubtful) out of 9 cases until the 28th day, but by the 60th day none of the individuals had detectable antibodies. It is evident that the serum interfered with the antigenic action of the vaccine when the negative 60-day specimens of this group are compared with 60-day specimens in Group Q (see Table IX).

[a] S5 was inoculated again with serum alone one year later, and once more showed no antibodies in the 2-, 6- and 10-day bleedings. Apparently some individuals (see also, for example, P5 in Table IV) bind or quickly eliminate those antibodies contained in the foreign protein of horse serum.

The following groups, QQ, RR, and QQRR, were set up to evaluate the "conditioning" of individuals (comprising groups Q and R) to a "booster" dose of the same vaccine. Observations published elsewhere[2] indicated that, occasionally, despite the absence of detectable antibodies following primary inoculation of HEP Flury, a response to a "booster" dose was noted.

Analysis of Table XI (Group QQ): *Six individuals from Group Q (Table IX) received a "booster" dose of 0.2 ml Flury HEP vaccine in one arm 6 months after the initial inoculation of the vaccine*

Compare with Table IX (Group Q). Antibody appeared in 1 out of 6 individuals by the 5th day, and relatively high levels of antibody were present by the 15th day in 4 out of 5 individuals tested. By the 30th day these levels dropped slightly but were present in 5 out of 6 individuals.

Analysis of Table XII (Group RR): *Seven individuals in Group R (Table X) received a "booster" dose of 0.2 ml Flury HEP vaccine in one arm 6 months after the initial inoculation of the vaccine*

Compare with Tables IX, X, and XI. At the time of the "booster" dose the serum of one of the individuals showed a doubtful level probably derived from the primary inoculation given 6 months previously. In the 30-day specimens following the "booster" dose little response was apparent. These results can be interpreted as indicating that the serum given with the primary vaccine inoculations 6 months previously interfered with the conditioning of the vaccine antigen so that, in contrast to Group QQ, no antibody recall was observed.

Analysis of Group QQRR: *Four new individuals previously uninoculated were inoculated intradermally with 0.2 ml of Flury HEP vaccine (to serve as controls for groups QQ and RR (Tables XI and XII)).*

All pre-inoculations and 30-day specimens were negative, indicating that a single inoculation of the vaccine produced no detectable antibodies in individuals who had not previously received preparatory sensitizing inoculations.

Discussion

Experimental arrangement

In the previous paper of this series,[1] to which the reader is referred, the limitations of experimental arrangement pertinent to that work were discussed at length. These limitations apply likewise to the work described in the present paper, particularly with respect to the performance and interpretation of the qualitative and quantitative tests used for determining the presence and level of antibodies. Much effort was given to developing a more sensitive test—the "modified" SN test described on page 915—and although comparative tests between the "modified" and "regular" test-methods showed a slight superiority of the former, the degree of difference was not great enough to warrant general adoption of the "modified" test for all specimens. However, by a slight rearrangement of the "regular" test, whereby practically undiluted serum (19 parts of serum to 1 of virus), instead of a 1:2 final dilution, was used for screening purposes, a greater sensitivity seems to have been achieved. Ten out of 12 sera which previously had negative results with the "regular" test gave positive reactions when tested with a minimal, instead of 1:2, final dilution of the serum.

In the previous series[1] all quantitative work was performed in one laboratory in order to keep at a minimum the important variable encountered when a different number of LD_{50} of virus was used in the neutralization test. This concentration of work in one laboratory was not found to be necessary in the present series because all the laboratories concerned had gained considerable experience with the tests used, and comparative tests of the same serum samples in different laboratories showed good consistency. Nevertheless, it is important to emphasise, as was done with the previous series, that numerical end-points of serum-neutralization, as recorded in the tables, must not be interpreted too strictly in comparing results in individuals or groups, because variations in the amount of virus (or the chance death or survival of one or two mice—a biological variable) may have caused some variation in the results.

Despite these inherent biological limitations, there is an obvious consistency of general level of antibody between serial specimens in individuals, between individuals in each group, between groups receiving similar treatment, and, in addition, between the two series. It is considered, therefore, that comparisons of efficacy of the different treatment schedules are valid on the following bases:

(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.

Interpretation of results

The relationship and significance of neutralizing antibody to protection against rabies infection is considered in our previous report.[1] The presence of *early and continued* antibody levels will be the criteria used here also for interpreting the results of the present series of experiments, with the added advantage over the first series of an observation period extending to 60 days instead of 28 days, to circumvent the possibility that 28-day antibodies in some of the groups were passive in nature.

Groups L and T (Tables I and V), which represent the usual course of vaccine treatment practised in many countries throughout the world, are used as a basis of comparison for the remaining groups. These groups, which received 14 daily inoculations of phenolized vaccine, showed antibodies, in most instances at a relatively high level, up to the 60th day—the last day of observation. It should be noted that other workers in comparable investigations reported persisting antibodies for from one to several years after the first inoculation of vaccine.[2, 7]

In Groups L and T (Tables I and V) and in comparable groups [1, 2] actively induced antibodies from daily inoculations of phenolized vaccine do not appear in many individuals until between the 10th and 15th day after the first inoculation of the vaccine. This gap can be filled by the administration of serum (Tables III, IV, VI-VIII, and our previous results).[1] Passive antibodies persisted in some individuals for as long as six weeks (see Table VIII). It is also noteworthy that two inoculations of serum do not appear to give antibody levels which are markedly superior to one inoculation (see Tables III, IV and VIII); in fact, as will be noted below, two inoculations of serum appear to be distinctly less advantageous than one.

In the present series clarification was sought on some of the results obtained in the first series, particularly with respect to the possible interference effect of serum on the antigenic action of the vaccine,[1] since actively induced antibodies are important for long-term protective effect after the passive antibodies from the antirabies serum have been eliminated.

Habel's studies in mice [4] showed that the interference effect of serum is a true phenomenon which could be quantitatively demonstrated in these animals, and that the interference with active antibody response to vaccine was paralleled by an interference with the production of resistance to virus challenge. His results also showed that "booster" doses of vaccine given after the 10th day of treatment are active in overcoming this interference.

Veeraraghavan et al.[5] studied the effect of varying doses of serum and phenolized vaccine in guinea-pigs before and after infection with street virus. His results indicated that serum at certain concentrations interfered with the protection conferred by the vaccine; an optimum relation between the dosage of serum and vaccine had to be maintained

for good results. Results were not proportionately better when larger doses of serum were given.

In antibody studies in human beings inoculated with HEP Flury vaccine and phenolized vaccine, with and without serum, Fox et al.[2] obtained further evidence that serum may exert a slight suppressive effect on active response to the vaccine, but they were of the opinion that with vaccines of full potency this effect would not be of practical significance.

Comparison of Tables II, III, and IV, Tables V, VI, and VII, and Tables IX, X, XI and XII indicates that serum exerted an interference effect on the antigenic action of both phenolized and HEP Flury vaccine when given in 3 inoculations 5 days apart. No such effect was observed when 14 inoculations of phenolized vaccine were given with one injection of serum, in contrast to 2 serum inoculations in the same combination where a distinct interference effect was observed. (It should be noted that in our first series [1] some interference from one inoculation of serum was seen with both the 7 daily and the 12 daily inoculations of vaccine.) Thus, in addition to the greater likelihood of unfavourable serum reactions with 2 inoculations, the second serum inoculation is further contra-indicated by its distinct interference effect.

It is of interest that phenolized vaccines of different potencies, according to the Habel test, were used in the first series,[1] the present series, and the experiments of Fox et al.[2] (protection against 70 000 LD_{50}, 1000-3000 LD_{50}, and 400 LD_{50} respectively). However, no clear-cut conclusions can be drawn as to the significance of this variable in serum-vaccine interference.

Results of studies by Fox et al.[2] and of those reported here with the reduced dosage schedules of phenolized vaccine (see Tables II-IV) and of Flury HEP vaccine intradermally (Tables IX-XII) give interesting leads for pre-exposure immunization of individuals, if not for post-exposure treatment.[a] The results indicate that such reduced schedules cannot, as yet, be substituted for the usual courses of vaccine at present given after exposure to rabies—that is, a minimum of 14 daily inoculations. Of great interest, however, is their applicability to pre-exposure immunization of individuals facing unusual and repeated hazards with respect to rabid animals, where multiple courses of vaccine and the consequent increased risk of paralytic accidents may be avoided. Group Q (Table IX), which showed relatively little antibody response to the primary course of 3 inoculations of Flury HEP vaccine given 5 days apart, responded to a single "booster" dose of the vaccine 6 months later in 5 out of 6 individuals (see Table XI). This corroborates other observations of the antigenic effect of a single "booster" dose of Flury HEP vaccine given to individuals who had received previous courses of treatment or preparation with either phenolized or Flury HEP vaccine at some period

[a] The results obtained by Fox et al.[2] with a four-dose schedule of vaccine given 5 days apart appear to be promising for post-exposure treatment.

varying between months and years previously.[8] The speed of antibody response to a "booster" inoculation of either HEP Flury or phenolized vaccine, and the possible use of serum with the "booster" dose, require further study.

TABLES I-VII. EXPLANATORY NOTE

These sera were examined by the "regular" SN test described in the text.

Qualitative tests made for screening purposes at a 1:2 final dilution of serum 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. Results recorded between parentheses and marked with an obelisk—( )†—are those obtained on selected serum specimens, virtually undiluted serum instead of final 1:2 dilution being used (see page 915).

Quantitative determinations are given as whole numbers representing the serum dilution (calculated according to the method of Reed and Muench) 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 carried out 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 tests recorded in the tables were carried out with 8-100 LD_{50} of virus.

TABLE I. GROUP L RESULTS *

Phenolized vaccine A: 14 daily inoculations (2 ml of 5% tissue suspension)

Individual	Days following first inoculation					
	0	10	15	21	28	60
L 1	—	—	8	20	63	3
L 2	—	—	—	2	4	2
L 3	—	—	9	19	32	NT
L 4	—	—	11	>125	>125	NT
L 5	—	—	12	>125	74	54
L 6	—	—	8	>125	>125	7
L 7	—	—	>125	>125	>125	>125
L 8	—	—	—	11	9	91
L 9	—	<5	12	>125	>125	96
L 10	—	—	6	80	103	66

* See *Explanatory Note* above. NT = not tested.

TABLE II. GROUP N RESULTS *

Phenolized vaccine A: 3 inoculations given on day 0, 5 and 10 (2 ml of 5% tissue suspension)

Individual	Days following first inoculation					
	0	10	15	21	28	60
N1	—	—	—	6	25	17
N2	—	—	—	—	—	—
N3	—	—	—	—	7	5
N4	—	—	—	>125	>125	74
N5	—	—	—	25	25	56
N6	—	—	—	2	—	2
N7	—	—	—	—	—	—
N8	—	5	—	—	—	6
N9	—	<5	8	48	38	10
N10	—	—	<5	<5	—	<5

* See *Explanatory Note* on page 924.

TABLE III. GROUP O RESULTS

Phenolized vaccine A: Same as N, plus 1 inoculation of serum (0.5 ml per kilogram body-weight) given at the same time as the first vaccine inoculation

Individual	Days following first inoculation						
	0	10	15	21	35	42	60
O1	—	3	2	2	2	—	—
O2	—	8	6	4	±	±	—
O3	—	11	6	—	2	±	—
O4	—	42	9	18	5	—	—
O5	—	18	5	5	±	±	—
O6	—	35	8	11	—	23	—
O7	—	11	2	2	±	±	±
O8	—	9	8	+	±	±	—
O9	—	23	20	16	13	5	<5
O10	—	29	11	14	13	8	6

* See *Explanatory Note* on page 924.

TABLE IV. GROUP P RESULTS*

Phenolized vaccine A: As in group O (see Table III), plus a second serum inoculation given on the 5th day (120 hours) after the first serum and vaccine inoculation

Individual	Days following first inoculation						
	0	10	15	21	28	42	60
P1	—	4	4	10	3	2	—
P2	—	(+)†	(+)†	—	—	—	—
P3	—	96	11	2	±	—	—
P4	—	13	8	±	<5	—	—
P5	—	—	(+)†	—	—	—	—
P6	—	19	20	10	±	—	—
P7	—	25	19	5	2	±	—
P8	—	32	6	2	—	—	—
P9	—	13	16	14	4	—	—
P10	—	29	40	17	9	6	5

* See *Explanatory Note* on page 924.

TABLE V. GROUP T RESULTS*

Phenolized vaccine B: 14 inoculations (0.5 ml of 20 % tissue concentration)

Individual	Days following first inoculation						
	0	10	15	21	28	42	60
T1	—	—	<5	12	21	23	16
T2	—	—	—	0	>125	120	69
T3	—	—	—	10	9	NT	13
T4	—	—	—	10	2	6	—
T5	—	24	>125	>125	>125	>125	69
T6	—	—	32	30	72	6	12

* See *Explanatory Note* on page 924. NT = not tested.

TABLE VI. GROUP U RESULTS*

Phenolized vaccine B: 14 inoculations (0.5 ml of 20 % tissue concentration) plus one serum (0.5 ml/kg), the serum given the same day as the first vaccine inoculation

Individual	Days following first inoculation						
	0	10	15	21	28	42	60
U1	—	(+)†	(+)†	<5	<5	—	—
U2	—	(+)†	(+)†	9	10	15	7
U3	—	(+)†	(+)†	14	41	125	74
U4	—	(+)†	(+)†	25	11	11	19
U5	—	16	16	16	88	78	12
U6	—	(+)†	—	—	30	22	16

* See *Explanatory Note* on page 924.

TABLE VII. GROUP V RESULTS*

As in group U (see Table VI), but with a second serum inoculation given five days after the first

Individual	Days following first inoculation						
	0	10	15	21	28	42	60
V1	—	<5	—	13	7	21	12
V2	—	14	10	8	<5	<5	—
V3	—	10	10	9	10	±	—
V4	—	±	±	—	—	±	±
V5	—	12	10	8	12	—	—
V6	—	18	8	12	4	—	—

* See *Explanatory Note* on page 924.

TABLE XI. GROUP QQ RESULTS

Six individuals from group Q received a "booster" dose of 0.2 ml Flury HEP vaccine in one arm 6 months after the initial inoculation of the vaccine

Individual	Days following "booster" inoculation			
	0	5	15	30
QQ2	0	0	NT	1.45
QQ3	0	0.10	2.45	1.55
QQ4	0	0.28	>2.55	1.35
QQ5	0	1.15	3.15	1.15
QQ7	0	0	0	0.45
QQ9	0	0	>1.50	>1.50

* See *Explanatory Note* on page 928. NT = not tested.

TABLE XII. GROUP RR RESULTS *

Seven individuals in group R received a "booster" dose of 0.2 ml Flury HEP vaccine in one arm 6 months after the initial inoculation of the vaccine

Individual	Days following "booster" inoculation			
	0	5	15	30
RR1	0.00	NT	NT	0.45
RR2	0	NT	NT	0
RR3	0	NT	NT	0.60
RR4	0	NT	NT	0
RR7	—	—	—	—
RR9	±	—	±	—
RR10	—	—	—	—

*See *Explanatory Note* on page 928. NT = not tested.

GROUP QQRR RESULTS

Four new individuals, to serve as controls for groups QQ and RR, were inoculated intradermally with 0.2 ml of Flury HEP vaccine. All pre-inoculations and 30-day specimens were negative.

RÉSUMÉ

Cette étude fait suite à des recherches sur les effets de la vaccination prophylactique antirabique et la persistance des anticorps passifs ou actifs chez des sujets qui n'ont été ni préalablement vaccinés ni mordus par des animaux enragés. Les auteurs ont suivi durant 60 jours la réponse sérologique au vaccin phéniqué, au vaccin de Flury HEP (nombreux passages sur œuf), et — dans certains cas — à l'injection vaccin + sérum, suivant diverses posologies. L'effet de doses de rappel de vaccin Flury, administrées 6 mois après la vaccination, a été étudié. Les résultats peuvent être résumés comme suit:

Une série de 14 injections quotidiennes de vaccin phéniqué a donné une réponse supérieure à celle que l'on obtient avec 3 injections à 5 jours d'intervalle.

Trois injections intradermiques de vaccin Flury HEP donnent une faible réponse, qui s'intensifie de façon satisfaisante à la suite d'une injection de rappel effectuée 6 mois plus tard.

La réponse à l'un ou l'autre des vaccins n'a été décelée, chez la plupart des sujets, qu'à partir du 10e-15e jour après la première injection.

Trois injections de vaccin phéniqué à 5 jours d'intervalle ont eu pour conséquence la production d'anticorps qui apparurent successivement chez les divers sujets à partir du 10e jour et qui étaient titrables chez 8 sur 10 d'entre eux le 60e jour.

Chez les sujets ayant reçu une injection de sérum antirabique, les anticorps passifs ont subsisté jusqu'à 42 jours. Une seconde injection de sérum n'a pas élevé le niveau des anticorps qu'avait suscité une première inoculation.

Une injection de sérum a supprimé la réponse sérologique à 3 injections de vaccin Flury par voie intradermique à 5 jours d'intervalle. Elle a également inhibé l'action stimulante de la dose de rappel.

En revanche, une injection de sérum n'a nullement affecté le résultat d'une série complète de vaccinations quotidiennes par le vaccin phéniqué. Mais deux doses de sérum ont eu une action inhibitrice indiscutable sur la production d'anticorps. L'action inhibitrice du sérum sur le pouvoir antigénique du vaccin, mise en évidence par de précédentes recherches, a donc été confirmée. Il faut souligner qu'elle ne se manifeste pas dans la posologie de 14 doses de vaccin + une injection de sérum.

Les résultats de cette étude présentent un intérêt particulier pour le traitement préventif — sinon curatif — de la rage. Une posologie réduite ne peut remplacer, pour les personnes mordues, la série de 14 vaccinations quotidiennes. Elle peut cependant être utile dans les cas de sujets exposés à de grands risques de morsures et à la répétition du traitement. On peut éviter dans ce cas la série complète d'injections de vaccin et les risques de complications qu'elle comporte. D'autre part, on a pu confirmer l'effet antigénique d'une dose de rappel unique de vaccin Flury HEP chez les individus ayant reçu des injections de vaccin phéniqué ou de vaccin Flury, des mois ou même des années auparavant. L'étude de ce problème sera poursuivie.

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TABLES VIII-XII. EXPLANATORY NOTE

These sera were examined by the " modified " SN test described in the text.

For specimens where only qualitative tests were performed, the results are represented by + (positive), — (negative), and ± (doubtful or trace), in a screening test carried out at a 1:100 dilution of the test serum-virus mixture. Positive indicates 4 or 5 out of 5 mice surviving, doubtful or trace indicates 2 or 3 survivals, and negative indicates 0 or 1 surviving. Further quantitative tests were not performed because it was considered that the information to be gained did not warrant the trouble.

Quantitative tests are indicated by numbers. These represent the log difference of LD_{50} titres observed between the negative control (normal or pre-inoculation serum plus virus) and test serum-virus mixtures. Thus, if the normal serum-virus mixture showed an LD_{50} titre (end-point) at a $10^{-5.60}$ dilution, and that of the test serum-virus mixture was $10^{-2.80}$, the result is given as 2.80, that is, the test serum neutralized $10^{2.80}$ LD_{50} of virus. A difference of 1.0 log or more is considered as indicative of specific antibody ; differences below 0.50 are considered negative.

TABLE VIII. GROUP S RESULTS *

Two groups of 5 individuals each, one group (S1-S5) receiving 1 serum inoculation, and second group (S6-S10) receiving 2 serum inoculations; second serum inoculation in latter group given 5 days (120 hours) after first serum inoculation

Individual	Days following first inoculation								
	0	2	6	10	15	21	28	42	60
S1	—	2.90	3.60	2.10	2.20	2.10	1.85	0.45	0.30
S2	—	3.15	3.85	3.40	1.70	2.20	2.00	1.90	0.70
S3	—	3.45	2.70	3.40	2.60	1.75	1.55	1.50	0
S4	—	+	±	+	+	+	NT	NT	NT
S5	—	+	—	—	—	±	—	—	—
S6	—	2.45	3.60	3.75	3.15	2.95	2.55	1.05	0.15
S7	—	2.55	3.15	2.60	3.15	2.60	2.15	1.75	0.70
S8	—	±	NT	NT	+	±	±	—	—
S9	—	+	+	+	+	+	+	+	—
S10	—	+	+	±	+	±	+	—	—

* See *Explanatory Note* above. NT = not tested.

TABLE IX. GROUP Q RESULTS *

Flury HEP vaccine intradermally as follows: 0.2 ml in each arm on day 0; 0.2 ml in one arm on day 5 (120 hours); 0.2 ml in second arm on day 10.

Individual	Days following first inoculation					
	0	10	15	21	28	60
Q1	0	0.15	0	0.50	0.05	1.05
Q2	0	0	0	0	0	0
Q3	0	0.25	0.70	0.35	0.45	0.70
Q4	0	0.70	0.05	0.65	0.65	0.35
Q5	0	0	0	0	0	1.10
Q6	—	±	±	NT	±	+
Q7	—	±	±	—	—	—
Q8	—	±	+	+	+	+
Q9	—	±	+	±	±	±
Q10	—	—	—	—	±	±

* See *Explanatory Note* on page 928. NT = not tested.

TABLE X. GROUP R RESULTS *

As in group Q (see Table IX) plus 1 serum inoculation given simultaneously with the first vaccine inoculation

Individual	Days following first inoculation					
	0	10	15	21	28	60
R1	0	2.80	2.65	1.95	2.00	0.45
R2	0	1.85	1.70	1.75	1.40	0
R3	0	2.10	2.20	1.60	1.20	0.25
R4	0	2.40	2.05	1.20	1.10	0.10
R5	0	2.35	1.55	1.05	1.55	0.25
R6	—	±	±	+	±	—
R7	—	—	+	±	+	—
R8	—	+	+	±	—	—
R9	Unsatisfactory — Pre-inoculation antibodies					
R10	—	—	±	—	—	—

* See *Explanatory Note* on page 928.