Bull. Org. mond. Santé / *Bull. Wld Hlth Org.* 1954, **10**, 781-788

ANTISERUM IN THE PROPHYLAXIS OF RABIES *

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SYNOPSIS

This paper reviews recent experimental evidence in support of inoculation with antirabies serum, the value of which, as a prophylactic measure, is compared with that of vaccine treatment alone. The author concludes that the chief advantages of combined antiserum-vaccine prophylaxis lie in the rapid treatment of cases who have suffered severe exposure, and in the possibility of reducing the incidence of post-vaccinal complications.

Although known for years as an experimentally feasible method of rabies prophylaxis, inoculation with antirabies serum has not been widely used as a routine public-health procedure. The fact that today serum is used in relatively few areas of the world, and prophylaxis with vaccine alone continues to be the usual method, is probably due to several causes. First of all, there has been a relative lack of quantitative experimental comparisons of the efficacy of serum with that of vaccine and, in fact, some of the earlier experiments showed serum to be ineffective. The case for serum has also suffered from the usual difficulty of evaluating any rabies prophylactic agent under field conditions in humans where incidence-rates are low, diagnostic criteria variable, reporting often irregular, and controls impossible. The relative lack of concern about, and little effort to eliminate, post-vaccinal paralysis, and the reluctance of practising physicians to change a method they have used apparently successfully for years, have also helped create this disinterest in rabies-serum prophylaxis.

It is obvious, however, that rabies should be one of the ideal diseases in which to use preventive antiserum. In how many other diseases do we know the exact time and location of the exposure, as we usually do in rabies ? The bite is frequently situated in an area where the antiserum can be directly applied to the site of exposure. Furthermore, very few infectious diseases have an incubation period as long as rabies. The only theoretical fact against the use of serum in rabies is that there is no definite viraemia stage required in the pathogenesis of the disease, and antiserum prophylaxis of virus diseases in general has been most useful in those diseases characterized by viraemia.

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

The experimental demonstration of the effectiveness of antirabies serum was first published by Babès & Lepp in 1889.[1] Since that time a number of workers have investigated this problem, with results varying from complete protection reported by Fermi[2] to no protection in Marie's experiments.[10] However, in more recent years Proca, Babès & Jonesco,[11] Shortt and co-workers,[12] Hoyt et al.,[5,6] Yen,[14] Koprowski et al.,[8,9] Smith,[13] Fermi, and our own laboratory [4] have obtained consistently good results in experimental animals, ranging from mice to monkeys. Many of the early results reported were based on too few experimental animals. The following data are from results of some of the better-controlled experiments.

Table I shows an experiment in guinea-pigs using a street virus challenge given into each masseter muscle, followed by prophylactic treatment begun 24 hours later. This is from the report of Koprowski, Van der Scheer & Black,[9] and shows the effectiveness of serum alone as compared to vaccine alone and suggests that the two combined may be even better.

TABLE I. COMPARATIVE PROTECTION OF GUINEA-PIGS TREATED WITH ANTISERUM OR VACCINE

Virus dilution	Type of protective treatment	Mortality ratio of guinea-pigs
1/160	Serum alone 1/8	4:10
	Serum (1/8) plus vaccine * . .	2:10
	Vaccine alone *	10:10
	Untreated controls	10:10

* 1/20 dilution of Semple-type vaccine injected in 0.5-ml amounts per animal for 14 consecutive days

Table II shows one of our experiments where street virus was given to guinea-pigs in both masseter muscles, and treatment with phenolized vaccine was started 24 hours later. Here again, as in the previous table, the unconcentrated rabbit serum alone gave more protection than vaccine alone and the combination of the two gave the best results. Here also it is shown that, even in an experiment where the control animals developed rabies on the 12th day, the start of vaccine could be delayed until the 4th day after a single dose of serum and still give excellent protection.

As the result of a number of experiments by several laboratories, it is now apparent that serum alone, even if given locally at the site of exposure, will not reduce the rabies mortality from that of the controls if an interval of longer than 72 hours has elapsed since virus exposure. This is an interesting correlation with experimental results on the distribution and multiplication of street virus after intramuscular inoculation. We and others have found [3,7] that virus could be demonstrated up to 72 hours afterwards in the

TABLE II. PROPHYLAXIS BY SERUM AND VACCINE, ALONE AND COMBINED

Treatment	Rabies/Total
Serum — 1 ml subcutaneously	6/10
Vaccine — 0.1 ml for 21 days	9/10
Serum and vaccine same day	3/10
Serum and vaccine 4 days later . . .	1/10
Controls	9/10

muscle at the site of the original inoculation, but also by this time it was already in the peripheral nerve and occasionally in the spinal cord. However, even under conditions in which serum would not prevent death from rabies, there is usually a definitely increased incubation period. Table III illustrates this in a guinea-pig experiment after intramuscular street-virus challenge, using unconcentrated rabbit serum and 5% phenolized vaccine. Of course, this prolongation of the incubation period is advantageous in giving sufficient time for active immunization with vaccine. The importance of this effect of serum is emphasized when one realizes that the majority of vaccine failures in humans occur in cases with short incubation periods.

TABLE III. EFFECT OF ANTIRABIES SERUM ON INCUBATION PERIOD

Interval : virus to treatment (days)		Mortality (%)	Incubation (days)
Serum— 2 ml sub-cutaneously	3	10	28
	24	30	34
	48	60	47
Vaccine— 10 daily 2-ml doses	3	60	13
	24	100	14
	48	80	13
Control	—	100	16

The persistence of virus locally at the site of its introduction for up to three days also adds to the significance of the local use of antiserum. Infiltration in and around the bite-wound with serum is a specific local treatment, and experimental evidence such as that shown in table IV illustrates the enhancement of the effectiveness of antiserum when used in this manner. Here a titration of fixed virus in the gastrocnemius muscle of mice was

TABLE IV. COMPARISON OF IMMUNE SERUM AT SITE OF CHALLENGE AND PERIPHERALLY

	Challenge virus dilutions (rabies/total)							50% end-point
	1/4	1/8	1/16	1/32	1/64	1/128	1/256	
Immune serum IM	0/4	0/5	0/4	0/5	0/4	0/5	0/5	<1/4
Immune serum IP	1/5	2/5	0/5	2/5	1/4	1/4	0/4	1/7
Normal serum IM	4/4	4/4	4/5	3/4	3/5	1/5	2/5	1/79
Control					4/5	2/5	0/5	1/103

Fixed virus IM — treatment immediately after challenge
Serum — 10 × concentrated rabbit 0.05 ml

followed by normal serum locally, antiserum locally, and antiserum intraperitoneally. Even though serum inoculated at a distance from the site of virus challenge gave good protection, when given locally it completely protected all mice at all dilutions of virus challenge.

Whenever antiserum is used in conjunction with antigen the question is always raised as to whether the presence of antibody will interfere with the immunizing capacity of the antigen, and this question has been asked concerning rabies serum when used with vaccine. In experiments in mice in our laboratory where a fixed virus was used intramuscularly with an incubation period of only seven days, comparison of serum followed by a course of vaccine begun the same day, with serum followed by vaccine started six days later, suggested that results were better at the longer interval. However, Koprowski working with street virus in hamsters was unable to show any difference in results with a one-day and a six-day interval. Table V, which is modified from Koprowski's unpublished experiments, gives some information on this point. In this experiment, hamsters received street virus intramuscularly, followed by various dilutions of antiserum given subcutaneously 24 hours later. Then, at an interval of five months, the survivors from this original experiment were re-inoculated intramuscu-

TABLE V. IMMUNITY TO RE-CHALLENGE IN HAMSTERS SURVIVING SERUM PROPHYLAXIS

Antiserum dilution	Original challenge *	Re-challenge **
1/2	7/65	32/51
1/32	31/65	3/34
1/128	55/67	3/12
Control	9/10	36/38

* 1/320 dilution street virus intramuscularly
** 1/10 dilution street virus intramuscularly five months after first challenge

larly with a more severe street virus challenge. It can be seen that with the more concentrated antiserum fewer animals succumbed to the original challenge, but on re-challenge most of the survivors were susceptible, whereas an intermediate quantity of antibody, while still giving moderate protection to the original challenge, gave excellent protection on re-challenge. Likewise, those receiving so little antibody that few survived the original challenge were also resistant to re-challenge. This would indicate that large amounts of antibody, while doing a good job of eliminating virus, also prevent its multiplication to the point where little antigenic activity remains, so that subsequently the animals are again susceptible when the passive immunity resulting from the antiserum is dissipated. However, when the amount of antibody is reduced to the point at which the original challenge breaks through and causes some rabies deaths, the survivors have had sufficient virus multiplication and antigenic stimulus for subsequent active immunity to be produced.

Table VI, again modified from Koprowski, gives some interesting information on this problem. Here a live virus vaccine which apparently depends on virus multiplication for its immunizing capacity was tested for its effect on the prophylactic value of antiserum. Street-virus-infected guinea-pigs received a single dose of serum and then groups received a single dose of Flury chick-embryo-type live-virus vaccine at 2 or 8 days' interval. Here there was obviously an antigenic effect from the single dose of live-virus vaccine, and results were no different when the vaccine was given two days or eight days after the serum.

TABLE VI. SERUM COMBINED WITH FLURY VACCINE : STREET VIRUS PROPHYLAXIS IN GUINEA-PIGS

Treatment		Mortality	Average day of death
serum	day of vaccine		
+	—	10/20	36
+	2	4/20	23
+	8	3/20	23
—	—	16/20	17

Challenge 1/320 dilution dog salivary gland intramuscularly
Serum — concentrated horse serum 1/2 dilution 0.25 ml subcutaneously
Vaccine — Flury avianized 0.25 ml 1/10 dilution intramuscularly

This whole question of the interval between the administration of antiserum and the start of the course of vaccine is a very practical one in many instances. Many times the diagnosis of rabies in the biting animal is not apparent at the time of a severe bite, and a period of observation is required to establish the presence of rabies, yet, particularly in areas where rabies

is not very prevalent, there may be hesitancy on the part of the physician in starting rabies vaccine immediately. Here antiserum can be administered and if subsequent events establish the diagnosis in the observed biting animal, vaccine may be started up to a week later, whereas if the animal continues in good health for a week it was probably incapable of transmitting rabies at the time of the bite and therefore vaccine is unnecessary. In this way, only a dose of serum has been received. In those areas where facilities are available for mouse inoculation techniques for establishing the presence of rabies virus in the brains of animals suspected of being rabid, and particularly in those brains that are negative for Negri bodies on microscopic examination, the interval of seven days after a dose of antiserum before deciding whether a course of vaccine is necessary is frequently sufficient to obtain positive results in the inoculated mice. Although there may be some question, from the experimental results cited, as to whether vaccine given after an interval following serum is *more* effective, at least the evidence indicates no decrease in the effectiveness of serum-vaccine prophylaxis as the result of this interval, which may offer some practical advantages.

The effectiveness of serum prophylaxis, either alone or combined with a course of vaccine, in humans following natural exposure has been demonstrated in several small series of cases. The reported results in the past have varied, but no uniformly potent serum has been in common use and in some instances the potency was unknown. The best results have been reported by Fermi, but when the only comparison that can be made is against a good vaccine, not much improvement can be demonstrated unless exposure is so severe that vaccine failures are frequent.

Interest in antirabies serum in the United States of America has come from another aspect of rabies prophylaxis over and above the demonstrated greater efficacy of serum-vaccine over vaccine alone in preventing the development of rabies after exposure. In spite of a fair amount of rabies in animals in the USA, relatively frequent exposures, and the administration of vaccine each year to as many as 50,000 people, the number of deaths from rabies is usually under 20, about half of which have never received adequate vaccine prophylaxis. In fact, by the accepted criteria, a large proportion of individuals receiving vaccine have not actually suffered an exposure and should not have received vaccine at all. With the administration of vaccine on this scale, post-vaccinal paralysis is bound to occur and in fact there is reason to believe that there are as many cases of this complication in our country each year as there are cases of rabies. We have therefore been greatly interested in any procedure which, while adequately protecting against rabies, would at the same time reduce the possibilities of post-vaccinal paralysis. One of the obvious ways to do this would be to eliminate completely the use of vaccine, and from the experimental results there is reason to believe that, in many less severe types of exposure, serum prophylaxis could be substituted for a course of vaccine. However,

this is a radical change from present methods; it might not be easily accepted by the medical profession and, in more severe exposures, might prove inadequate. Therefore, the introduction of antiserum has been looked upon as a means of reducing the number of doses of vaccine, in the hope that by so doing the incidence of post-vaccinal complications will also be reduced. There is good reason to believe that most post-rabies vaccine paralysis is caused by an organ-specific type of allergic reaction to the brain tissue in vaccine, and the multiple antigen stimulus which takes place daily in the course of vaccine prophylaxis is the important determinant of such complications. Since most cases of vaccine paralysis occur late in, or after, the course of vaccine, reducing the number of doses should help reduce its incidence. In the USA a single dose of concentrated antiserum produced in horses in the dose of 0.5 ml per kg of body-weight, followed by 14 daily doses of vaccine for severe exposures, is currently becoming available. Emphasis will be placed on the local infiltration of the bite-wound where practicable. Serum, of course, as with vaccine alone, should be administered as early as possible, but even later than 72 hours after exposure it may prolong the incubation period sufficiently for vaccine to produce active immunity in time to prevent the clinical disease.

In conclusion, then, there is a definite need—particularly in certain areas of the world—for a more effective procedure for preventing rabies after severe exposures, where the incubation period may be too short for vaccine alone to be effective, and where it is desirable to develop a method which might reduce the incidence of post-vaccinal paralysis. The use of antiserum, combined with a course of vaccine based on experimental and field trials, offers a possible answer to these questions, and has now reached the point where it can be recommended for more routine use.

RÉSUMÉ

La séroprophylaxie antirabique n'a guère été appliquée jusqu'à maintenant comme mesure courante de santé publique. Pourtant la rage est l'une des maladies où elle pourrait donner les meilleurs résultats, puisque l'on connaît le point d'infection — où le sérum peut être directement appliqué — et le temps d'incubation. Théoriquement, le seul obstacle à l'usage du sérum est l'absence, au cours de la pathogenèse de la rage, d'un stade net de virémie, stade auquel, dans les autres viroses, le sérum est particulièrement actif.

On a démontré à l'évidence que la sérothérapie n'est d'aucune utilité lorsqu'elle est appliquée — même au point d'infection — plus de 72 heures après la morsure infectante. Mais, même dans les cas où il n'a pas empêché la mort, le sérum a augmenté le temps d'incubation, ce qui, dans des conditions favorables, donne au vaccin administré après morsure, le temps de susciter une résistance active de l'organisme. Le fait que le virus persiste jusqu'à trois jours au point de pénétration favorise l'application locale efficace du sérum.

Lorsque le sérum est utilisé avec le vaccin, on s'est demandé si les anticorps du sérum n'exerçaient pas une action empêchante sur le pouvoir immunisant du vaccin. L'expérience a montré que les résultats sont en effet meilleurs si un certain laps de temps — jus-

qu'à six jours — s'écoule entre l'administration du sérum et celle du vaccin. D'autres observations ont prouvé également que de fortes quantités d'anticorps, en éliminant partiellement le virus vaccinal, n'en laissent que des doses trop faibles pour susciter une immunité durable, de sorte que l'animal vacciné peut devenir de nouveau réceptif, lorsque l'immunité conférée par le sérum a disparu. Le sérum présente l'avantage de pouvoir être administré en attendant la confirmation éventuelle du diagnostic, dans les cas où il n'est pas évident que l'animal mordant soit enragé.

Aux Etats-Unis, l'emploi de sérum antirabique présente un autre intérêt encore. Malgré la présence de la rage chez un grand nombre d'animaux et de morsures fréquentes, les décès par rage ne dépassent pas une vingtaine annuellement, et la moitié des victimes n'ont pas reçu de sérum à titre prophylactique. Quelque 50.000 personnes sont vaccinées annuellement et une forte proportion de ce nombre ne sont jamais mordues et n'auraient pas dû être vaccinées. Des accidents de paralysie postvaccinale auraient ainsi pu être évités. Aussi porte-t-on un grand intérêt à la séroprophylaxie qui, tout en protégeant contre la rage, diminue les risques de paralysie. Il semble même que dans les cas de morsures peu graves, la sérothérapie pourrait remplacer une dose de vaccin. La sérothérapie peut être envisagée comme moyen de diminuer le nombre des doses vaccinales, et par conséquent, les risques d'accidents paralytiques.

En conclusion, la nécessité existe dans certains pays du monde, d'un procédé plus efficace de protection contre la rage, à la suite de morsures graves, lorsque la période d'incubation, très courte, empêche le vaccin d'être actif assez tôt, et là où il s'agit de réduire les risques de paralysie postvaccinale. Le sérum antirabique, combiné à une dose de vaccin, remplit les conditions requises par ces cas, et il a été actuellement mis au point de façon à pouvoir être recommandé pour un emploi plus général.

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