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Value of Antirabies Vaccine With and Without Serum against Severe Challenges

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Earlier studies with antirabies serum and vaccine have been extended to determine the value of serum, vaccine, or serum and vaccine combined against rabies challenges of increasing degrees of severity. While serum alone was not found to have any protective effect, vaccine alone was sufficient against mild challenges, the superiority of combined therapy with both serum and vaccine becoming evident at a challenge of about 50 LD_{50}. With challenges of over 300 LD_{50} no treatment was of any value. It was also found that with a recommended optimum dose of serum, the usual vaccine dose could be halved—a matter of importance in countries with a high incidence of neuroparalytic accidents following administration of nervous tissue vaccine.

Employing challenges varying from 1.3 to 16.8 LD_{50} of the NYC, Marimuthu and Masuli strains of street virus Veeraraghavan et al. (1957) [3] reported that (*a*) antirabies serum alone, given before or after infection, while definitely prolonging the incubation period, had no saving effect; (*b*) with doses of 5% Semple vaccine comparable to those administered to human beings it was possible to confer solid protection to animals against virulent strains of street virus provided the treatment was started 7 days before challenge; and (*c*) combined therapy with serum and vaccine given after infection was of great value under certain conditions and that there appeared to be an optimum relationship between the quantity of serum given and the antigenicity and dosage of vaccine administered. It has also been reported by Veeraraghavan (1959)[4] that by pooling batches of vaccine from several infected sheep brains it was possible to produce a vaccine which was superior in antigenicity to the NIH (United States National Institutes of Health) Reference Vaccine 155-D and that the pooled vaccine conferred a significant protection to guinea-pigs challenged with virulent strains of street virus, even when administered an hour after infection.

Studies were continued to determine the value of (*a*) serum, (*b*) pooled vaccine and (*c*) serum and pooled vaccine against severe challenges. Experiments were also undertaken to find out the extent to which the dosage of pooled vaccine could be reduced when given in combination with varying doses of serum against such challenges.

The results of these investigations are summarized below.

MATERIALS AND METHODS

Animals

Healthy guinea-pigs, bred at the Pasteur Institute of Southern India and weighing about 400 g, were used in all protection experiments. The mice employed for serum neutralization tests were bred at the Institute from the Rockefeller Institute strain.

Challenge virus

Apart from the NYC and Marimuthu strains used in the earlier work, a jackal strain (J. 154/56) of street virus was used extensively in this study. The material employed was the lyophilized submaxillary gland suspension of a jackal infected in nature. The method of administering the challenge and of determining the number of LD_{50} used in each experiment was the same as that described by Veeraraghavan et al. (1957—*op. cit.*).

Antirabies serum

Lederle's antirabies serum L, 1000 units per 10 ml, was used in one experiment and antirabies serum

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[3] Veeraraghavan, N., Balasubramanian, A. & Subrahmanyan, T. P. (1957) *Bull. Wld Hlth Org.*, 17, 943

[4] Veeraraghavan, N. (1959) *Bull. Wld Hlth Org.*, 20, 121

PIC in the rest. The dosage of serum administered and the method of determination of the neutralizing antibody content of the serum against the particular challenge were the same as those described earlier (*op. cit.*). Serum was always given intramuscularly. It was found that against the same challenge the neutralizing antibody content of serum PIC was about five times greater than that of serum L.

Vaccine

Pooled vaccine obtained by mixing 12 batches of 5% Semple vaccine prepared from 12 sheep brains, according to the method described by Veeraraghavan (1959—*op. cit.*), was used. Vaccine was always given subcutaneously.

Period of observation

Guinea-pigs were observed for a minimum period of six months and mice for 30 days.

RESULTS

It has been reported by Veeraraghavan et al. (1957) that using a total dose of 2.1 ml of vaccine, corresponding to a Class III (severe exposure) dosage of 140 ml for a person weighing 70 pounds or more, and serum L, the best results were obtained when one-fifth of the dosage of serum recommended (0.25 ml per pound of body-weight) was employed. Using the same batch of vaccine it was found that one-thirtieth of the dosage of serum PIC gave the best results. The fact that there was some survival among animals given 2.1 ml of vaccine in fourteen equal doses of 0.15 ml even when the treatment was started 1 hour after challenge indicated that it was better to administer the vaccine in fourteen doses instead of seven. The results, however, were not statistically significant. On the other hand, later work (Veeraraghavan, 1959) showed that excellent protection could be obtained against similar challenges with half the dose (14×0.075 ml) of pooled vaccine. Statistically significant protection could also be demonstrated with 14×0.15 ml of pooled vaccine against higher challenges up to 105 LD_{50} of the J.154/56 strain of virus.

Experiment 1

An experiment was undertaken to determine the nature of protection afforded against a moderately severe challenge (100 LD_{50}) by serum L or PIC, pooled vaccine and a combination of either of the sera and pooled vaccine administered in doses similar to those reported earlier against mild challenges.

The challenge virus (J. 154/56) was the lyophilized submaxillary gland suspension of a jackal infected in nature. The different lines of treatment tried were: (*a*) 0.25 ml of serum L or PIC given locally into the leg muscles 1 hour after challenge; (*b*) 14 doses of 0.15 ml of vaccine, started 1 hour after challenge; and (*c*) 0.25 ml of 1 : 5 serum L or 1 : 25 serum PIC together with 14 doses of 0.15 ml of vaccine, started 1 hour after challenge. Also an attempt was made to determine the effectiveness of the different lines of treatment when the same challenge was given into the neck muscles instead of into the gastrocnemius muscles. The results are summarized in Table 1.

It was found that with the same challenge the LD_{50} in animals challenged into the neck was 109 compared with 101 for those challenged into the leg. The average incubation period in the former was 14.6 days compared with 16.6 days in the latter.

The administration of 0.25 ml of serum L or PIC locally into the gastrocnemius muscles 1 hour after a challenge with 101 LD_{50} of virus conferred no protection, the mortality in either case being 100%.

Pooled vaccine, 14×0.15 ml, started 1 hour after challenge, conferred significant protection when the challenge was given into the leg but not when it was administered into the neck muscles.

Combined therapy with 0.25 ml of 1 : 5 dilution of serum L or 1 : 25 dilution of serum PIC and 14×0.15 ml of vaccine started 1 hour after challenge gave very good protection when the challenge was given into the leg or neck muscles. The survival rates were better when the challenge was given into the neck muscles.

In the above experiment an attempt was also made to determine the value of combined therapy started 1 hour, 3 days and 6 days after challenge. The results are presented in Table 2.

Treatment with 0.25 ml of 1 : 25 serum PIC and 14×0.15 ml of vaccine started 1 hour after a challenge with 101 LD_{50} of J. 154/56 virus into the leg muscles conferred very good protection. The same dose of serum PIC and vaccine gave significant protection even when treatment was started after 3 days. But if the treatment was delayed for 6 days the protection was not always significant.

Combined therapy with 0.25 ml of 1 : 25 serum PIC and 14×0.15 ml of vaccine started 1 hour after a challenge with 101 LD_{50} of virus given into the leg muscles gave significantly better protection than

TABLE 1

RABIES SERUM AND VACCINE AGAINST SEVERE CHALLENGES INTO THE NECK AND THE LEG

Challenge J.154/56		Serum [a]			Pooled vaccine [a] dosage	Mortality	Average incubation period (days)	Serum ND [b]
Site	LD_{50}	Serum used	Dilution	Dose (ml)				
Leg	101	L	1 : 5	0.25	14 × 0.15 ml	7/25	20.7	112
Leg	101	L	neat	0.25 local	nil	25/25	27.0	562
Leg	101	—	—	nil	14 × 0.15 ml	18/25	14.4	—
Leg	101	—	—	nil	nil	25/25	16.6	—
Neck	109	L	1 : 5	0.25	14 × 0.15 ml	5/25	18.4	112
Neck	109	—	—	nil	14 × 0.15 ml	22/25	14.0	—
Neck	109	—	—	nil	nil	25/25	14.6	—
Leg	101	PIC	1 : 25	0.25	14 × 0.15 ml	8/25	17.0	126
Leg	101	PIC	neat	0.25 local	nil	25/25	37.0	3 162
Leg	101	—	—	nil	14 × 0.15 ml	18/25	14.4	—
Leg	101	—	—	nil	nil	25/25	16.6	—
Neck	109	PIC	1 : 25	0.25	14 × 0.15 ml	2/25	12.5	126
Neck	109	—	—	nil	14 × 0.15 ml	22/25	14.0	—
Neck	109	—	—	nil	nil	25/25	14.6	—

[a] Started 1 hour after challenge.
[b] ND = neutralizing doses against challenge virus.

when the same treatment was started 3 or 6 days later. It was also better than treatment with vaccine alone.

There was no significant difference in the protection conferred by 0.25 ml of 1 : 25 serum PIC and 14 × 0.15 ml of vaccine started 3 or 6 days after challenge and vaccine alone started 1 hour after challenge, which also gave significant protection.

Combined therapy with 0.25 ml of 1 : 25 serum PIC and 14 × 0.15 ml of vaccine started 1 hour after a challenge with 109 LD_{50} of virus into the neck muscles conferred excellent protection. The protection obtained with the same dosage of serum and vaccine started 3 or 6 days after challenge, though not equally good, was statistically significant.

Treatment with serum PIC and vaccine started 1 hour after a challenge with 109 LD_{50} of virus into the neck muscles gave significantly better protection than the same treatment started 3 or 6 days later. The protection obtained when the treatment was started 3 or 6 days after challenge did not seem to differ. While the protection conferred by the serum and vaccine therapy started 3 days after challenge was better than that obtained with vaccine alone, started 1 hour after challenge, it was not so when the same treatment was delayed for 6 days.

Experiment 2

In this experiment the effect of giving half the dose of vaccine, keeping the serum dosage constant, against different challenges of the Marimuthu strain of virus was investigated. The results obtained with 0.25 ml of 1 : 25 dilution of serum PIC and 14 doses of 0.15 ml or 0.075 ml of vaccine against 9.4, 18.8, 37.6 and 75.2 LD_{50} of Marimuthu strain of virus are summarized in Table 3.

Fourteen doses of 0.15 ml or 0.075 ml of vaccine gave significant protection against 9.4 LD_{50} of virus. The protection was not significant against the higher challenges.

Combined therapy with serum and 14 doses of 0.15 ml or 0.075 ml of vaccine conferred significant protection against all the challenges employed.

No significant difference in protection could be demonstrated between treatment with serum and 14 doses of 0.15 ml or 0.075 ml of vaccine or either dose of vaccine given alone against 9.4 LD_{50} of virus.

Against 18.8 LD_{50} of virus, serum combined with 14×0.15 ml of vaccine conferred a significant protection compared with that afforded by 14×0.15 ml of vaccine alone. Against higher challenges there was generally no significant difference in the protection afforded by combined therapy with serum and 14 doses of 0.15 ml or 0.075 ml of vaccine compared with that obtained by vaccine treatment alone.

There was no significant difference in the protection conferred by 14 doses of 0.15 ml or 0.075 ml of vaccine and serum against any of the challenges employed.

Experiment 3

In view of the finding that there was no significant difference in the protection afforded by serum and

TABLE 2

RABIES SERUM AND VACCINE AGAINST SEVERE CHALLENGES INTO THE NECK AND THE LEG AT VARIOUS INTERVALS AFTER CHALLENGE

Challenge J. 154/56		Serum PIC			Pooled vaccine		Mortality	Serum ND [a]
Site	LD_{50}	Time started	Dilution	Dose (ml)	Time started	Dosage		
Leg	101	1 hour after challenge	1 : 25	0.25	1 hour after challenge	14 ×0.15 ml	8/25	126
Leg	101	3 days after challenge	1 : 25	0.25	3 days after challenge	14 ×0.15 ml	18/25	126
Leg	101	6 days after challenge	1 : 25	0.25	6 days after challenge	14 ×0.15 ml	20/25	126
Leg	101	—	—	nil	1 hour after challenge	14 ×0.15 ml	18/25	—
Leg	101	—	—	nil	—	nil	25/25	—
Neck	109	1 hour after challenge	1 : 25	0.25	1 hour after challenge	14 ×0.15 ml	2/25	126
Neck	109	3 days after challenge	1 : 25	0.25	3 days after challenge	14 ×0.15 ml	15/25	126
Neck	109	6 days after challenge	1 : 25	0.25	6 days after challenge	14 ×0.15 ml	19/25	126
Neck	109	—	—	nil	1 hour after challenge	14 ×0.15 ml	22/25	—
Neck	109	—	—	nil	—	nil	25/25	—

[a] ND = neutralizing doses against challenge virus.

TABLE 3

RABIES SERUM AND VACCINE AGAINST DIFFERENT CHALLENGES OF MARIMUTHU STRAIN

Challenge Marimuthu LD_{50}	Serum PIC[a]		Pooled vaccine[a] dosage	Mortality	Serum ND[b]
	Dilution	Dose (ml)			
9.4	1 : 25	0.25	14 × 0.15 ml	5/20	168
9.4	1 : 25	0.25	14 × 0.075 ml	4/20	168
9.4	—	nil	14 × 0.15 ml	3/10	—
9.4	—	nil	14 × 0.075 ml	2/10	—
9.4	—	nil	nil	10/10	—
18.8	1 : 25	0.25	14 × 0.15 ml	2/20	84
18.8	1 : 25	0.25	14 × 0.075 ml	5/20	84
18.8	—	nil	14 × 0.15 ml	8/10	—
18.8	—	nil	14 × 0.075 ml	6/10	—
18.8	—	nil	nil	10/10	—
37.6	1 : 25	0.25	14 × 0.15 ml	7/19	42
37.6	1 : 25	0.25	14 × 0.075 ml	8/20	42
37.6	—	nil	14 × 0.15 ml	6/10	—
37.6	—	nil	14 × 0.075 ml	8/10	—
37.6	—	nil	nil	10/10	—
75.2	1 : 25	0.25	14 × 0.15 ml	10/20	21
75.2	1 : 25	0.25	14 × 0.075 ml	9/20	21
75.2	—	nil	14 × 0.15 ml	8/10	—
75.2	—	nil	14 × 0.075 ml	10/10	—
75.2	—	nil	nil	10/10	—

[a] Started 1 hour after challenge.

[b] ND = neutralizing doses against challenge virus.

14 doses of 0.15 ml or 0.075 ml of vaccine against different challenges of the Marimuthu strain of virus, the experiment was repeated using the J. 154/56 strain. The results obtained with 0.25 ml of 1 : 25 dilution of serum PIC and 14 doses of 0.15 ml or 0.075 ml of vaccine against 46, 92 and 184 LD_{50} of the virus are presented in Table 4.

Fourteen doses of 0.15 ml or 0.075 ml of vaccine conferred significant protection against 46 LD_{50} of

TABLE 4

RABIES SERUM AND VACCINE AGAINST DIFFERENT CHALLENGES OF J. 154/56 STRAIN

Challenge J. 154/56 LD_{50}	Serum PIC[a]		Pooled vaccine[a] dosage	Mortality	Serum ND[b]
	Dilution	Dose (ml)			
46	1 : 25	0.25	14 × 0.15 ml	4/20	168
46	1 : 25	0.25	14 × 0.075 ml	8/20	168
46	—	nil	14 × 0.15 ml	13/20	—
46	—	nil	14 × 0.075 ml	14/20	—
46	—	nil	nil	19/20	—
92	1 : 25	0.25	14 × 0.15 ml	3/20	84
92	1 : 25	0.25	14 × 0.075 ml	4/20	84
92	—	nil	14 × 0.15 ml	17/20	—
92	—	nil	14 × 0.075 ml	19/20	—
92	—	nil	nil	19/20	—
184	1 : 25	0.25	14 × 0.15 ml	11/20	42
184	1 : 25	0.25	14 × 0.075 ml	13/20	42
184	—	nil	14 × 0.15 ml	18/20	—
184	—	nil	14 × 0.075 ml	19/20	—
184	—	nil	nil	19/20	—

[a] Started 1 hour after challenge.

[b] ND = neutralizing doses against challenge virus.

virus. The protection was not significant with either dose of vaccine against the other challenges employed.

There was no significant difference in the protection conferred by 14 doses of 0.15 ml or 0.075 ml of vaccine against 46, 92 and 184 LD_{50} of virus.

Combined therapy with serum and 14 doses of 0.15 ml or 0.075 ml of vaccine gave good protection against 46 and 92 LD_{50} of virus. The protection against 184 LD_{50} was only moderate but significant.

Combined therapy with serum and either dose of vaccine always gave significantly better protection than treatment with either dose of vaccine alone against all the challenges.

The protection afforded by serum and 14×0.15 ml of vaccine was not significantly different from that conferred by the same dose of serum and 14×0.075 ml of vaccine against 46, 92 and 184 LD_{50} of virus.

The protection conferred by serum and 14×0.15 ml of vaccine against 46 and 92 LD_{50} of virus was not significantly different. But in both cases it was significantly better than the protection obtained against 184 LD_{50} of virus. Such a clear difference, however, could not be demonstrated with the same dose of serum and 14×0.075 ml of vaccine.

Experiment 4

In this experiment the effect of varying the doses of vaccine and serum PIC was studied. Table 5 shows the results of administering (*a*) 0.25 ml of neat (undiluted), or 1 : 12.5 and 1 : 25 dilutions of serum with 14×0.15 ml of vaccine; (*b*) 0.25 ml of 1 : 25 dilution of serum with 14 doses of 0.075 ml or 0.038 ml of vaccine; and (*c*) 0.25 ml of 1 : 50 dilution of serum with 14 doses of 0.075 ml or 0.038 ml of vaccine.

There was no significant difference in the protection conferred by 14×0.15 ml of vaccine and 0.25 ml of neat, or 1 : 12.5 or 1 : 25 dilutions of serum. While all the treatments gave significant protection compared to the untreated controls the results of combined therapy were not better than those obtained with vaccine alone.

With 0.25 ml of 1 : 25 dilution of serum there was no significant difference between the protection afforded by 14 doses of 0.15 ml, 0.075 ml or 0.038 ml of vaccine, although the difference between 14 doses of 0.075 ml and 0.038 ml was nearly significant.

With 0.25 ml of 1 : 50 dilution of serum, 14×0.075 ml of vaccine gave significantly better protection than 14×0.038 ml.

TABLE 5

DIFFERENT DOSES OF RABIES SERUM AND VACCINE AGAINST A SEVERE CHALLENGE

Challenge J. 154/56 LD_{50}	Serum PIC[a]		Pooled vaccine[a] dosage	Mortality	Serum ND[b]
	Dilution	Dose (ml)			
105	neat	0.25	14×0.15 ml	8/25	3162
105	1 : 12.5	0.25	14×0.15 ml	9/25	253
105	1 : 25	0.25	14×0.15 ml	8/25	126
105	1 : 25	0.25	14×0.075 ml	4/25	126
105	1 : 25	0.25	14×0.038 ml	11/25	126
105	1 : 50	0.25	14×0.075 ml	5/25	63
105	1 : 50	0.25	14×0.038 ml	14/25	63
105	—	nil	14×0.15 ml	11/25	—
105	—	nil	nil	25/25	—

[a] Started 1 hour after challenge.
[b] ND = neutralizing doses against challenge virus.

Experiment 5

In view of the interesting results obtained in the previous experiment with 0.25 ml of 1 : 25 dilution of serum PIC and 14×0.038 ml of vaccine and 0.25 ml of 1 : 50 dilution of serum and 14×0.075 ml of vaccine, the value of these two schedules of treatment started 1 hour and 1, 2, 3 and 6 days after challenge was investigated. The results are summarized in Table 6.

When treatment was started 1 hour after infection, administration of (*a*) 0.25 ml of 1 : 25 dilution of serum and 14×0.038 ml of vaccine, (*b*) 0.25 ml of 1 : 50 dilution of serum and 14×0.075 ml of vaccine as well as (*c*) 14×0.075 ml of vaccine alone, gave significant protection compared with the untreated controls.

When treatment was started 1, 2, 3 and 6 days after infection, treatment with 0.25 ml of 1 : 50 dilution of serum and 14×0.075 ml of vaccine conferred no advantage over 14×0.075 ml of vaccine alone.

With the moderate challenge employed delay in starting the treatment by 1, 2, 3 or 6 days did not

TABLE 6

TWO DIFFERENT DOSES OF RABIES SERUM AND VACCINE AT VARIOUS INTERVALS AFTER INFECTION

Challenge J.154/56 LD_{50}	Serum PIC			Pooled vaccine		Mortality	Serum ND [a]
	Time started	Dilution	Dose (ml)	Time started	Dosage		
64	1 hour after challenge	1 : 25	0.25	1 hour after challenge	14 × 0.038 ml	8/19	225
64	1 hour after challenge	1 : 50	0.25	1 hour after challenge	14 × 0.075 ml	5/19	112
64	—	—	nil	1 hour after challenge	14 × 0.075 ml	10/20	—
64	—	—	nil	—	nil	20/20	—
64	1 day after challenge	1 : 25	0.25	1 day after challenge	14 × 0.038 ml	6/20	225
64	1 day after challenge	1 : 50	0.25	1 day after challenge	14 × 0.075 ml	8/20	112
64	—	—	nil	1 day after challenge	14 × 0.075 ml	12/20	—
64	2 days after challenge	1 : 25	0.25	2 days after challenge	14 × 0.038 ml	6/20	225
64	2 days after challenge	1 : 50	0.25	2 days after challenge	14 × 0.075 ml	11/20	112
64	—	—	nil	2 days after challenge	14 × 0.075 ml	11/19	—
64	3 days after challenge	1 : 25	0.25	3 days after challenge	14 × 0.038 ml	9/20	225
64	3 days after challenge	1 : 50	0.25	3 days after challenge	14 × 0.075 ml	11/20	112
64	—	—	nil	3 days after challenge	14 × 0.075 ml	12/20	—
64	6 days after challenge	1 : 25	0.25	6 days after challenge	14 × 0.038 ml	12/20	225
64	6 days after challenge	1 : 50	0.25	6 days after challenge	14 × 0.075 ml	9/20	112
64	—	—	nil	6 days after challenge	14 × 0.075 ml	12/20	—

[a] ND = neutralizing doses against challenge virus.

TABLE 7
EFFECT OF INCREASING THE DOSE OF SERUM ON THE DOSE OF VACCINE

Challenge J. 154/56 LD_{50}	Serum PIC [a]		Pooled vaccine [a] dosage	Mortality	Serum ND [b]
	Dilution	Dose (ml)			
57	1 : 2	0.1	14 × 0.019 ml	17/20	1 360
57	1 : 10	0.1	14 × 0.019 ml	15/20	272
57	—	nil	14 × 0.019 ml	19/20	—
57	1 : 2	0.1	14 × 0.038 ml	10/20	1 360
57	1 : 10	0.1	14 × 0.038 ml	14/20	272
57	—	nil	14 × 0.038 ml	19/20	—
57	1 : 2	0.1	14 × 0.075 ml	11/19	1 360
57	1 : 10	0.1	14 × 0.075 ml	10/20	272
57	—	nil	14 × 0.075 ml	14/20	—
57	—	nil	nil	20/20	—

[a] Started 1 hour after challenge.
[b] ND = neutralizing doses against challenge virus.

produce a significant lowering in the protection conferred by any of the schedules of treatment.

Experiment 6

In this experiment the effect of reducing the dosage of vaccine further and increasing the dose of serum was studied. The value of 0.1 ml of 1 : 2 or 1 : 10 dilution of serum PIC along with 14 doses of 0.019 ml, 0.038 ml or 0.075 ml was tried. The results are presented in Table 7.

It was found that with 0.1 ml of 1 : 2 or 1 : 10 dilution of serum and 14 × 0.019 ml of vaccine or vaccine alone there was no significant protection. The administration of a higher dose of serum made no significant difference.

With 0.1 ml of 1 : 2 or 1 : 10 dilution of serum and 14 × 0.038 ml of vaccine, it was found that the higher dose of serum combined with vaccine gave a significantly better result than vaccine alone. But the protection conferred by either dose of serum and vaccine was not significantly different. The vaccine alone afforded no protection.

With 14 × 0.075 ml of vaccine, it was found that the administration of 0.1 ml of either 1 : 2 or 1 : 10 dilution of serum made no significant difference. Both the combined treatments as well as the vaccine alone gave significant protection compared with the untreated controls.

Experiment 7

In the previous experiment the challenge proved to be only 57 LD_{50}. The value of 0.25 ml of 1 : 25 dilution of serum PIC together with 14 doses of 0.019 ml, 0.038 ml and 0.075 ml of vaccine was investigated against a higher challenge. The results are summarized in Table 8.

It was found that none of the vaccine schedules gave protection.

Combined therapy with 0.25 ml of 1 : 25 dilution of serum and vaccine gave better results than vaccine alone when the dosages employed were 14 doses of 0.019 ml, 0.038 ml or 0.075 ml.

Experiment 8

In this experiment the value of 0.1 ml of 1 : 10 dilution of serum PIC and 14 doses of 0.019 ml, 0.038 ml or 0.075 ml of vaccine against severe challenges was tried. The results are presented in Table 9.

TABLE 8
RABIES SERUM AND DIFFERENT DOSES OF VACCINE AGAINST SEVERE CHALLENGE

Challenge J. 154/56 LD_{50}	Serum PIC [a]		Pooled vaccine [a] dosage	Mortality	Serum ND [b]
	Dilution	Dose (ml)			
103	1 : 25	0.25	14 × 0.019 ml	13/20	59
103	—	nil	14 × 0.019 ml	18/20	—
103	1 : 25	0.25	14 × 0.038 ml	11/20	59
103	—	nil	14 × 0.038 ml	19/20	—
103	1 : 25	0.25	14 × 0.075 ml	9/20	59
103	—	nil	14 × 0.075 ml	18/20	—
103	—	nil	nil	19/19	—

[a] Started 1 hour after challenge.
[b] ND = neutralizing doses against challenge virus.

TABLE 9
RABIES SERUM AND DIFFERENT DOSES OF VACCINE AGAINST INCREASINGLY SEVERE CHALLENGES

Challenge J. 154/56 LD_{50}	Serum PIC[a]		Pooled vaccine[a] dosage	Mortality	Serum ND[b]
	Dilution	Dose (ml)			
576	1 : 10	0.1	14 × 0.038 ml	20/20	27
576	1 : 10	0.1	14 × 0.075 ml	19/20	27
288	1 : 10	0.1	14 × 0.019 ml	15/20	54
288	1 : 10	0.1	14 × 0.038 ml	13/20	54
288	1 : 10	0.1	14 × 0.075 ml	12/20	54
144	1 : 10	0.1	14 × 0.019 ml	15/20	108
144	1 : 10	0.1	14 × 0.038 ml	9/20	108
144	1 : 10	0.1	14 × 0.075 ml	6/20	108
144	—	nil	14 × 0.075 ml	16/20	—
144	—	nil	nil	20/20	—

[a] Started 1 hour after challenge.
[b] ND = neutralizing doses against challenge virus.

It was found that when the challenge was 576 LD_{50}, there was no protection with serum and 14 doses of 0.038 ml or 0.075 ml of vaccine.

When the challenge was 288 LD_{50}, combined therapy with serum and 14 × 0.019 ml did not confer significant protection. But results were significant when serum was given with 14 doses of 0.038 ml or 0.075 ml of vaccine.

When the challenge was 144 LD_{50}, serum plus 14 doses of 0.038 ml or 0.075 ml of vaccine gave significant protection. The results were not significant with serum and 14 × 0.019 ml of vaccine or vaccine alone.

The protection afforded by serum and 14 × 0.075 ml and not 14 × 0.038 ml of vaccine was significantly better than treatment with serum and 14 × 0.019 ml of vaccine.

The administration of serum improved significantly the results of treatment with 14 × 0.075 ml of vaccine.

DISCUSSION

In this study an attempt has been made in guinea-pigs to assess the value of (*a*) vaccine, (*b*) serum and (*c*) serum and vaccine against severe challenges and to determine the optimum conditions under which combined serum and vaccine treatment would give the best results. Also large-scale experiments have been undertaken to determine the extent to which our present dosage schedule of vaccine could be reduced when combined with serum therapy.

Based on the results of vaccine treatment alone in the different experiments, it may be possible to arrive at the minimum dose of vaccine necessary to give significant protection when administered after infection. The results with varying doses of vaccine against different challenges of J. 154/56 strain of virus are summarized in Table 10.

It was found that the protection conferred by the same dose of vaccine against nearly equal challenges varied from experiment to experiment. For instance, while significant protection was conferred by 14 × 0.15 ml of vaccine against 101 LD_{50} of virus in experiment 1 and 105 LD_{50} in experiment 4, the protection was not significant against 92 LD_{50} in experiment 2. Thus while pooled vaccine conferred significant protection against mild challenges, the results were not so regular at higher levels of challenge. It was, nevertheless, found that 14 × 0.075 ml of vaccine seemed to be an adequate dose against challenges up to about 50 LD_{50} while 14 × 0.15 ml appeared to be more efficacious at challenges nearing 100 LD_{50}. This finding would emphasize the need of administering Class III dosage (14 × 10 ml for persons weighing 70 pounds and above) in cases of severe exposure, particularly when serum is not given.

Antirabies serum L or PIC alone administered locally, while definitely prolonging the incubation period, had no saving effect when the challenges were about 100 LD_{50} (experiment 1). This finding is the same as that reported earlier with lower challenges.

With combined therapy it was found that when the challenge was mild, about 10 LD_{50}, the administration of serum in addition to vaccine did not appear to be of advantage (experiment 2).

When the challenge was about 50 LD_{50} (experiments 2, 3, 5, 6) the protection conferred by combined therapy with serum and vaccine was better than that obtained with vaccine alone, though not always statistically significant.

With challenges of about 100 LD_{50}, vaccine did not give consistently significant protection even

TABLE 10

POST-INFECTION TREATMENT WITH DIFFERENT DOSES OF RABIES VACCINE AGAINST SEVERE CHALLENGES

Experiment No.	Challenge J. 154/56 LD_{50}	Pooled vaccine[a] dosage	Mortality
4	46	14 × 0.15 ml	13/20
4	46	14 × 0.075 ml	14/20
7	57	14 × 0.019 ml	19/20
7	57	14 × 0.038 ml	19/20
7	57	14 × 0.075 ml	14/20
6	64	14 × 0.075 ml	10/20
4	92	14 × 0.15 ml	17/20
4	92	14 × 0.075 ml	19/20
1	101	14 × 0.15 ml	18/25
8	103	14 × 0.019 ml	18/20
8	103	14 × 0.038 ml	19/20
8	103	14 × 0.075 ml	18/20
5	105	14 × 0.15 ml	11/25
1	109	14 × 0.15 ml	22/25
9	144	14 × 0.075 ml	16/20
4	184	14 × 0.15 ml	18/20
4	184	14 × 0.075 ml	19/20

[a] Started 1 hour after challenge.

when administered in 14×0.15 ml doses but combined therapy with serum and vaccine always gave very good protection (experiments 1, 3, 4, 7).

Against challenges of 144 LD_{50} (experiment 8) and 184 LD_{50} (experiment 3) vaccine treatment was of no value while combined therapy with serum and vaccine gave good protection.

When the challenge was 288 LD_{50} (experiment 8) the protection with combined therapy was still significant while at 576 LD_{50} there was no protection.

In the majority of experiments a dose of 0.25 ml of 1 : 25 dilution of serum PIC per pound of body-weight or its equivalent, 0.1 ml of 1 : 10 dilution, which gave good results earlier, was used and gave regularly good results (experiments 1 to 8). When the dose of serum was reduced by half while the dose of vaccine remained constant the results were found to be equally good (experiment 4). But subsequent work has shown that the protection is not consistent. With serum L, a dose of 0.25 ml of 1 : 5 dilution gave good results (experiment 1).

Attempts were made to determine the value of increasing the dosage of serum on the effective dose of vaccine to be administered. It was found (experiment 4) that with 14×0.15 ml of vaccine there was no advantage in giving more than 0.25 ml of 1 : 25 dilution of serum PIC against a challenge of 105 LD_{50} of virus. With 0.25 ml of 1 : 25 dilution of serum there was no significant difference in the protection afforded by 14 doses of 0.15 ml or 0.075 ml of vaccine (experiments 2, 3, 4). The results with 14×0.038 ml of vaccine and the above dose of serum were, however, poorer (experiment 4). The results of experiment 6 employing a challenge of 57 LD_{50} also indicated that even when the dosage of serum was increased fivefold, there was no protection with 14×0.019 ml of vaccine. But 14 doses of 0.038 ml or 0.075 ml of vaccine gave equally good results with 0.1 ml of 1 : 2 or 1 : 10 dilution of serum. These results indicated that there was no advantage in giving a higher dose of serum and that the vaccine dosage could not be reduced beyond a certain minimum.

With different doses of vaccine it was found that, when administered with serum, there was very little difference in the protection conferred by 14 doses of 0.15 ml or 0.075 ml of vaccine (experiments 2, 3, 4). When the vaccine dosage was reduced to 14×0.038 ml the survival rate was generally lower (experiments 4, 6, 7, 8). On further reduction of the vaccine dose to 14×0.019 ml, the protection was definitely poorer (experiments 6, 7, 8). These findings suggested that with 0.25 ml of 1 : 25 dilution of serum or its equivalent, a safe minimum dosage of vaccine would appear to be 14×0.075 ml per pound of body-weight. Thus by administering serum it would seem possible to halve the dosage of vaccine. This dosage confers protection even against challenges where vaccine alone

is ineffective. These findings are of great importance to countries where the incidence of neuroparalytic accidents following vaccine therapy is high.

When combined therapy was started at varying intervals after infection it was found that the best results were obtained when the treatment was started 1 hour after challenge. By delaying the treatment the advantage conferred by serum was generally lost (experiments 1, 5).

An interesting observation in experiment 1 was that the survival rate was better with combined therapy when the challenge was given into the neck muscles instead of into the leg muscles, although the average incubation period in the former group was shorter and the mortality among those treated with vaccine alone was greater.

Another important finding was that with any of the doses of serum and vaccine employed, 100% protection could not be obtained against the different challenges tested.

CONCLUSIONS

1. Results of post-infection treatment with vaccine against varying challenges indicated that, when vaccine alone was given, a dosage of 14×0.15 ml per pound of body-weight—corresponding to a Class III (severe exposure) dosage of 140 ml for a person weighing 70 pounds or more—was necessary.

2. Antirabies serum L or PIC alone administered locally 1 hour after challenge conferred no protection.

3. There was no advantage in giving serum when the challenge was mild as vaccine alone gave good protection in such cases.

4. When the challenge was about 100 LD_{50} the protection conferred by combined therapy was very good and markedly superior to that obtained with vaccine treatment alone.

5. Against challenges ranging from 100 to 200 LD_{50} the protection conferred by combined therapy was moderately good while vaccine treatment was of no value.

6. When the challenge was about 300 LD_{50} the protection conferred by combined therapy was still significant, while at higher challenges no treatment was of any value.

7. There was no advantage in giving high doses of serum. Also, it did not seem possible to reduce the dosage of vaccine beyond a certain limit by increasing the dose of serum.

8. With the dose of serum PIC recommended (0.25 ml of 1 : 25 dilution per pound of body-weight) it was found possible to reduce the dosage of vaccine by half (14×0.075 ml per pound of body-weight).

9. Delay in starting treatment resulted in the loss of the advantage conferred by serum in combined therapy.

10. With the doses of serum and vaccine employed it was not possible to obtain 100% protection against any of the challenges tested.

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RÉSUMÉ

Le traitement contre la rage, au moyen de vaccin, de sérum, ou de sérum + vaccin a été mis à l'épreuve sur le cobaye infecté expérimentalement par le virus rabique (souches NYC, Marimuthu et Masuli). Dans leurs conclusions, les auteurs indiquent que le vaccin assure une bonne protection en cas d'infection légère, et que sérum est alors superflu. Le sérum seul, administré localement une heure après l'infection d'épreuve, ne confère aucune protection. Lorsque l'inoculation correspond à 100 DL_{50}, la thérapie vaccin + sérum est excellente, et nettement supérieure au vaccin seul. De 100-200 DL_{50}, les résultats du traitement combiné sont médiocres, et ceux du vaccin seul sont nuls. Au-delà de 300 DL_{50}, aucun traitement n'est efficace. Il n'y a pas avantage à administrer de fortes doses de sérum. Il n'est pas possible non plus de réduire la dose de vaccin au-dessous d'une certaine limite, en augmentant celle du sérum. Toutefois, on peut réduire de moitié la dose de vaccin nécessaire, en administrant une dose calculée de sérum, correspondant à 0,25 ml d'une dilution à 1 : 25 par livre de poids corporel. Ce fait a de l'importance dans les régions où l'administration de tissu nerveux cause fréquemment des accidents neuroparalytiques.