13619

Protective Value of Antiviral Serum in Experimental Rabies Infection.

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Recently Webster[1] has shown by animal protection test the inefficiency of most commercial rabies vaccines. Although the problem of finding a more satisfactory vaccine has attracted the attention of various investigators, it is as yet not fully solved. In view of this, it seems worthwhile to reëxamine the possible value of sero-prophylaxis of which contradictory results were reported by earlier workers.[2, 3, 4] While more recently Hoyt and coworkers[5] have demonstrated the protective action of antiviral serum in white mice, their results only showed an increase in the percentage of survivals over the untreated controls. Jonnesco[6] also has reported the prolongation of the incubation period of 3 out of 5 guinea pigs previously treated with antiviral serum. In the light of such suggestive but inconclusive results, it seems desirable to test more carefully the efficacy of antirabies serum with control of the important experimental factors such as weight and age of test animals, dosage of virus, route of infection, quantity of serum in antiviral units given, and the interval between infection and antiserum administration. In this communication observations indicating the beneficial effects of a single dose of antiviral serum against multiple fatal doses of rabies infection are presented.

Materials and Method. A strain of fixed rabies virus (Habel)* maintained by frequent intracerebral passages in mice was used. Antiviral sera were prepared from rabbits by repeated intraäbdominal inoculations of 5 cc doses of a phenol-treated vaccine given twice weekly for the first 4 weeks followed by similar injections with a live vaccine for 4-6 weeks. The live vaccine is a 10% brain emulsion of

1 Webster, L. T., *J. Exp. Med.*, 1939, **70**, 87.

2 Fermi, C., *Centralbl. f. Bakt.*, 1909, **52**, 576.

3 Kraus, R., and Holobut, T., *Z. f. Immunitätsforsch. u. exp. Therap.*, 1909, **3**, 130.

4 Marie, A., *Ann. Inst. Pasteur*, 1908, **22**, 271.

5 Hoyt, A., Fisk, R. T., and Tracy, R. L., *J. Inf. Dis.*, 1936, **59**, 152.

6 Jonnesco, D., *Compt. rend. Soc. Biol.*, 1939, **130**, 1145.

* This strain of virus originally obtained from Dr. Habel, National Institute of Health, Washington, D.C., has been kindly supplied to us by the Squibb Institute for Medical Research, N.J.

rabbit dying on 8-10th day of infection. The phenol-treated vaccine is a similar emulsion containing 0.5% phenol which has been incubated at 37°C for 48 hours. All vaccines were used within 2 weeks after preparation. The potency of antiviral serum is expressed in antiviral units. One antiviral unit is the minimal amount of serum contained in the 0.03 cc mixture of serum and virus which after incubating at 37°C for one hour, suffices to inactivate 1,000 intracerebral M.L.D. of the fixed virus for Swiss mice weighing 10-12 g (5-6 weeks old). One intracerebral M.L.D. is the minimum amount of virus given in 0.03 cc that will cause death of 50% of inoculated animals. One intramuscular M.L.D. is the minimal amount of virus in 0.1 cc which when injected into the left gastrocnemius is just sufficient to cause death of 50% of injected animals. Since the number of M.L.D. contained in the same virus suspension varies somewhat depending on the size of animals tested, we have employed animals of approximately the same age and weight for both control and test groups in each experiment. Sera containing 3.3×10^4 antiviral units were usually obtained and were kept in frozen state in the refrigerator without any preservative. Normal rabbit serum regularly showed no antiviral power.

Effect of Antiviral Serum on Intracerebral Infection. Normal Swiss mice weighing 15-16 g were divided into groups A. B. and C. 0.03 cc from each 10-fold dilution (10^{-1} to 10^{-7}) of the fixed virus suspension was given intracerebrally to 6 animals from each group. Within 10-15 minutes following the inoculation, each of the animals in group B received intraäbdominal injection of 0.5 cc normal rabbit serum diluted 1:3 in saline and those of group C, 0.5 cc of antiviral serum (1:3) containing 10^4 antiviral units. Group A animals were not treated. These animals were then observed for a period of 6 weeks. Table I records day of death of these animals. From this, it is obvious that the amount of antiserum given exerted a favorable effect by showing survival of a few animals with marked prolongation of lives of all the rest of the treated animals as compared with the untreated controls or those treated with normal serum.

That the prolongation of the life-span is induced regardless of dosage of infection ranging from 10 to 10^6 M.L.D. is difficult to explain. It is possible that the prolonged course of disease is caused by the small amount of virus which before coming in contact with antiserum, has already entered the nerve cells. The free virus remaining at the site of injection was probably inactivated by the absorbed antiviral serum.

TABLE I.
Effect of Antiviral Serum on Intracerebral Infection.

Treatment	Virus Dilution 1:10	1:10²	1:10³	1:10⁴	1:10⁵	1:10⁶	1:10⁷
A Control	*5,5,5, 5,6,6	5,6,6, 6,7,7	6,6,6, 7,7,7	6,6,7, 7,8,9	7,7,7, 7,8,8	7,8,8, 8,8,9	10,11,S, S,S,S
B Normal serum	5,5,6, 6,6,6	6,6,7, 7,7,7	7,7,7, 7,7,7	7,7,7, 7,7,7	7,7,8, 8,8,8	8,9,9, 9,9,10	9,10,11, S,S,S
C Immune serum	11,11,16, 16,17,(S)	10,13,14, 16,17,(S)	12,13,13, 14,16,S	9,12,13, 14,15,16	(2),5,14, 14,16,(S)	14,15,16, 16,17,S	—

TABLE II.
Effect of Antiviral Serum on Intramuscular Infection.

Treatment	Serum dilution	Antiviral units	Virus Dilution 1:10	1:40	1:160	1:640	1:2,560	1:10,240
I None		0	7,8,8, 8,8,9	8,8,8, 9,9,10	8,8,9, 9,9,10	(1),8,9, 9,10,11	9,9,10, 10,10,11	11,12,S, S,S,S
II Normal serum	1:1	0	8,8,8, 9,9,9	8,8,8, 8,9,10	8,8,9, 9,10,10	8,8,8, 8,9,10	(2),9,10, 10,10,10	11,12,14, S,S,S
III Immune serum	1:1	1.6×10^4	(2),8,27, (S),S,S	12,(S),(S), S,S,S	22,S,S, S,S,S	S,S,S, S,S,S	S,S,S, S,S,S	—
IV " "	1:4	4×10^3	11,11,12, 22,26,27	15,18,(S), S,S,S	8,S,S, S,S,S	S,S,S, S,S,S	S,S,S, S,S,S	—
V " "	1:10	1.6×10^3	10,10,13, 13,14,(S)	12,12,13, 16,17,(S)	9,10,11, 14,S,S	12,S,S, S,S,S	S,S,S, S,S,S	—
VI " "	1:100	1.6×10^2	—	—	9,9,10, 10,12,14	9,10,17, 18,(S),S	10,S,S, S,S,S	S,S,S, S,S,S

*The numerals denote day of death following infection.
(S) Animals showing symptoms of paralysis but surviving over 6 weeks.
S Animals surviving over 6 weeks without showing symptoms.
(1) and (2) Animals dying from undetermined cause.

Effect of Antiviral Serum on Intramuscular Infection. Six groups of Swiss mice weighing 10-12 g were each given 0.1 cc of serial 4-fold dilutions of the fixed virus into the left gastrocnemius. Within 10-15 minutes after inoculation, 0.5 cc of undiluted normal rabbit serum was injected intraäbdominally to each of the animals in Group II, and similarly, 0.5 cc of antiserum undiluted, 1:4, 1:10, and 1:100 in saline were separately given to animals in Groups III, IV, V, and VI respectively. Group I animals were left untreated. Table II summarizes observations on these animals. It will be seen that the antiviral serum diluted as high as 1:100 (1.6×10^2 antiviral units) was only able to protect a majority of the animals against 4 intramuscular M.L.D. But with the increase of the antiviral units given, protection against more intramuscular M.L.D. was obtained. The beneficial effect afforded by the antiserum against intramuscular infection is far better than against the intracerebral infection.

Vaccine and Antiviral Serum on Intracerebral Infection. Since in our hands, vaccination of animals with phenol-treated vaccine according to Habel's[7] procedure of potency test gave only partial protection for animals infected with highly fatal doses, the following experiment was carried out to determine the combined effect of the antiviral serum and vaccine treatment. Animals weighing 10-12 g were divided into 2 groups, vaccinated and unvaccinated, of 18 animals each. In the vaccinated group, all the animals were given intraabdominal injections of 0.25 cc of a 0.5% phenol-treated vaccine on alternate days for 6 injections. Fourteen days after the first injection, all the animals in both groups were each given intracerebrally 0.03 cc (100,000 intracerebral M.L.D.) of the virus. Immediately following virus inoculation, 0.5 cc of 1:3 normal rabbit serum was given intraäbdominally to 6 animals from both groups, and 0.5 cc of antiserum 1:3 (5×10^3 antiviral units) was given separately to another 6 animals from both groups. The remaining 6 animals from both groups were left untreated. Table III compares the results observed with normal serum- and immune serum-treated animals. The untreated controls showed no essential difference from the normal serum treated groups, and are not tabulated. This table clearly shows that whereas animals receiving vaccine and normal serum gave only 50% survival, those receiving vaccine and antiserum all survived over 6 weeks without showing symptoms. The unvaccinated animals receiving antiserum alone showed prolongation of the life-span of animals, confirming the results obtained above. The

[7] Habel, K., *Public Health Reports*, 1940, **55**, 1473.

TABLE III.
Effect of Vaccine and Antiviral Serum on Intracerebral Infection.

	Vaccinated	Non-vaccinated
Normal serum	8,8,11,S,S,S	6,6,6,7,7,7
Immune serum	S,S,S,S,S,S	10,13,15,15,16,17

satisfactory protection afforded by the combined vaccine and antiserum treatment as compared with the administration of either alone is of interest. Further studies along this line are in progress to explore in greater detail its practicability.

Conclusion. Administration of a single dose of potent antiviral serum intraäbdominally 10-15 minutes after infection is capable of prolonging the life-span of the intracerebrally infected animals, and completely saving the lives of some of the intramuscularly infected animals depending on the dosage of infection and amount of antiviral units given. The combined use of vaccine and antiserum afforded full protection to the animal against 100,000 intracerebral M.L.D. of the virus.

13620

Effect of Malonate and Iodoacetate on Respiration of Brains of Rats of Various Ages.

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It has been shown that the oxygen uptake of the excised brain of the infant rat is much lower than that found in the adult.[1, 2] Furthermore, during the development of the brain of this animal 3 levels in its metabolism can be distinguished.[3] The lowest level is found during the first week of life; the highest occurs between the fourth and seventh week; the third, or adult level, is reached at about 20 weeks, and is significantly lower than the second level. Parallel changes are found to occur also in the rate of glucose utilization. In contrast to this, the glycolytic activity during the first week is rela-

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1 Himwich, H. E., Baker, Z., and Fazekas, J. F., *Am. J. Physiol.*, 1939, **126**, 601.

2 Tyler, D. B., and van Harreveld, A., *Am. J. Physiol.*, 1941, **133**, 472.

3 Tyler, D. B., and van Harreveld, A., in press.