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The Duration of Immunity in Dogs Following the Single-Injection Method of Anti-Rabic Vaccination

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THE DURATION OF IMMUNITY IN DOGS FOLLOWING THE SINGLE-INJECTION METHOD OF ANTI-RABIC VACCINATION*

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The control of rabies in animals by immunization has received renewed consideration as a result of the development of the single-injection method of vaccination. In man, immunization against rabies has been widely and successfully practiced since the work of Pasteur.

The propagation and dissemination of rabies in man and animals depend primarily on its occurrence in the dog and the control of the disease in this species of animal will to a large extent prevent its spread. Any method for the immunization of dogs that will be generally used should be free of the danger of producing rabies in the treated animal, as the development of the disease would be extremely dangerous on account of the close association of dog with man.

Pasteur's experiments (1) showed that the virus of rabies in naturally infected animals, called street virus, was not constant in virulence, since the disease developed at varying periods of time following the inoculation of such a virus. Later a virus of constant virulence was obtained by successive passages thru rabbits. This virus, called fixed virus, has an incubation period of from five to seven days. A progressive attenuation of the fixed virus by drying over caustic potash at a temperature of from 20 to 23 degrees C. resulted in the virus being avirulent after fourteen days.

Pasteur first immunized dogs and then applied his treatment to human beings. The usual treatment in man requires from fourteen to twenty-one injections. This method of immunization has been used only in a limited way in animals and obviously is not a practical procedure.

The number of injections necessary to protect animals was reduced to six in the modified Högyes method, which consists in using dilutions of unattenuated fixed virus. This method has been rather extensively used in treating exposed animals, however, it has never been generally employed in the control of rabies.

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Favorable results were reported in Japan by Umeno and Doi (2), using a single injection of an attenuated virus for the prophylactic vaccination of dogs against rabies. This procedure appears to have value as a practical means of preventing the spread of the disease. The results reported by Eichhorn and Lyon (3) in the United States on the use of a similar method of vaccination, confirms the work of the Japanese investigators.

Experiments conducted by Schoening (4), of the United States Department of Agriculture, also indicate that, at least in some instances, there is a high degree of immunity produced by this method of vaccination. However, against one strain of street virus the vaccine conferred only slight protection. This suggests that more than one strain of rabic street virus exists in this country.

A rabic virus, obtained from the brains and cords of dogs infected with fixed virus, was killed and used by Schlingman (5) as an anti-rabic vaccine for dogs. This vaccine is reported as protecting treated dogs when they were exposed to an infection of fixed virus.

The results obtained by various investigators with the single-injection method of vaccination, while not in complete agreement, are of such significance as to warrant further efforts to determine the possibilities of this treatment.

The application of this method, as a practical measure for controlling rabies in dogs, is being seriously considered by dog owners, veterinarians, and public health officials. In some localities compulsory vaccination of all dogs is being attempted in an effort to control the spread of the disease. For these reasons experimental work was undertaken to obtain additional information regarding the value of the single-injection method of immunization against rabies.

OBJECT OF EXPERIMENTS

The purpose of these experiments was to determine the duration of immunity following a single-injection of anti-rabic vaccine and its efficacy in protecting against street virus from different sources. To accomplish this a vaccine was prepared according to the method of Umeno and Doi and used to immunize dogs. After a period of six months these dogs were injected with different strains of virus.

EXPERIMENTAL ANIMALS

For this experiment dogs were secured from a municipal dog pound and represented various breeds and ages. They ranged in weight from thirteen to thirty-four pounds, the majority weighing

between twenty and thirty pounds. Indivdual kennels were provided and so placed as to prevent the dogs' coming in contact with one another. These dogs were confined in a manner that permitted a limited amount of exercise.

All dogs intended for vaccination were held under observation for a period of three to five months. This allowed them to become accustomed to their environment and was considered a sufficient period of time for the incubation of most diseases to which they might have been exposed.

THE VACCINE AND ITS ADMINISTRATION

The vaccine used in these experiments was prepared according to the method suggested by Umeno and Doi, with a slight modification, the vaccine being held at 37 degrees C. for seventy-two hours instead of room temperature or in a refrigerator for fourteen to thirty days.

In this method the brain and spinal cord of rabbits, in which rabies has developed in seven days following the injection of fixed virus, are ground together. Phenolized glycerin water is added to the mass in the ratio of four parts to one of the brain and cord substance. The phenolized glycerin water consists of sixty parts glycerin and forty parts water containing 1.25 percent phenol. The dose of vaccine was 5 cc. for each dog, equally distributed in four subcutaneous injections.

EXPERIMENT I

In the first experiment, forty-three dogs were used. Twenty-seven of these dogs were vaccinated December 30, 1926 and the remaining sixteen were used as controls. The controls were assumed to be non-vaccinated dogs and had been under observation for more than a month.

Approximately six months after vaccination these dogs were classified into Groups "A", "B", and "C". Each group had nine vaccinated dogs and six, five, and five controls, respectively.

The viruses used to infect the dogs consisted of the brain substance of dogs that had died of rabies, the diagnosis having been confirmed by the finding of Negri bodies. These cases of rabies were obtained from three widely separated sections of Ohio. The brains were held in glycerin six to eight weeks prior to use. The entire brain was ground in a mortar with sterile salt solution, using approximately one part of brain to five of the diluent. The suspension was then strained thru several layers of cheesecloth.

Two methods of injecting the virus were used, designated as intraocular and traumatic. In the intraocular the virus was injected into the anterior chamber of the eye, while in the traumatic an effort was made to lacerate a superficial nerve of the inner surface of the thigh by several thrusts of the needle. However, it is probable that the traumatic injection represents little more than a subcutaneous or intramuscular inoculation.

In the intraocular method 0.2 cc. and in the traumatic 0.3 cc. of the brain suspension was injected, using a syringe with a 26 gauge needle. Five of the vaccinated dogs in each group were inoculated with virus by the intraocular and four by the traumatic method. Three controls in each group received intraocular injections of the virus, while in Group A three dogs and in B and C two each were inoculated traumatically.

DISCUSSION

The inability to standardize rabic street virus makes it difficult to establish a satisfactory dosage for infecting dogs in experimental work. The ideal dose of virus would be just the amount necessary to cause the disease in susceptible animals. The intraocular method of infection while considered to be drastic, has been employed by most investigators, including Umeno and Doi, Eichhorn and Lyon, Reichel and Schneider, and Schoening.

In an effort to establish a means of artificial infection that more nearly approaches natural exposure, an attempt was made to lacerate a nerve trunk at the seat of inoculation. To accomplish this, several thrusts were made with the hypodermic needle in the region of a superficial nerve and the virus injected. By this mode of procedure it was hoped that an infection could be produced which would be similar to the bite of a rabid dog. However, the results of this test show the fallacy of the plan, since no dog, either vaccinated or control, developed rabies from this method of injection.

Various clinicians have observed a marked difference in the number of cases of rabies that develop following the bite of different rabid dogs. In some instances rabies develops in nearly all of the exposed subjects, while in others only a few show evidence of the disease. The work reported by Schoening suggests that there is more than one strain of rabic street virus in the United States. For these reasons virus from outbreaks of rabies in different localities were used in this experiment.

TABLE 1—Rabies Immunization Tests 6 Months After Vaccination

GROUP A

Dog	Weight	Vaccinated	Amount injected	Street virus* injected intraocularly	Amount	Street virus* injected traumatically	Amount	Results
No.	*Lb.*	*Date*	*cc.*	*Date*	*cc.*		*cc.*	
1	22	December 30, 1926	5	July 8, 1927	0.2			Died September 7, 1927 R.†
2	20	December 30, 1926	5	July 8, 1927	.2			Died July 28, 1927 I.‡
3	23	December 30, 1926	5	July 8, 1927	.2			Died July 30, 1927 R.
6	30	December 30, 1926	5	July 8, 1927	.2			Died August 4, 1927 R.
7	33	December 30, 1926	5	July 8, 1927	.2			Died August 9, 1927 R.
8	25	December 30, 1926	5			July 8, 1927	0.3	Alive October 7, 1927
9	24	December 30, 1926	5			July 8, 1927	.3	Alive October 7, 1927
10	21	December 30, 1926	5			July 8, 1927	.3	Alive October 7, 1927
12	34	December 30, 1926	5			July 8, 1927	.3	Alive October 7, 1927
4	17	Control		July 8, 1927	.2			Alive October 7, 1927
5	22	Control		July 8, 1927	.2			Died July 30, 1927 R.
34	20	Control		July 8, 1927	.2			Died July 26, 1927 R.
35	23	Control				July 8, 1927	.3	Alive October 7, 1927
36	13	Control				July 8, 1927	.3	Alive October 7, 1927
43	18	Control				July 8, 1927	.3	Alive October 7, 1927

*Brain of rabid dog placed in glycerin May 13, 1927.
†R Died of rabies—confirmed by demonstration of Negri bodies.
‡I Negative for rabies as determined by microscopical examination of brain and intracranial inoculation of rabbits.

TABLE 2.—Rabies Immunization Tests 6 Months After Vaccination

GROUP B

Dog	Weight	Vaccinated	Amount injected	Street virus* injected intraocularly	Amount	Street virus* injected traumatically	Amount	Results
No.	*Lb.*	*Date*	*cc.*	*Date*	*cc.*	*Date*	*cc.*	
13	21	December 30, 1926	5	July 8, 1927	0.2	....	.....	Alive October 7, 1927
14	27	December 30, 1926	5	July 8, 1927	.2	.....	.....	Alive October 7, 1927
15	27	December 30, 1926	5	July 8, 1927	.2	.....	.....	Alive October 7, 1927
16	31	December 30, 1926	5	July 8, 1927	.2	.....	.....	Alive October 7, 1927
17	15	December 30, 1926	5	July 8, 1927	.2	....	.....	Alive October 7, 1927
18	33	December 30, 1926	5	....	.....	July 8, 1927	0.3	Alive October 7, 1927
20	28	December 30, 1926	5	....	.... ..	July 8, 1927	.3	Alive October 7, 1927
21	17	December 30, 1926	5	... .	.. ..	July 8, 1927	.3	Alive October 7, 1927
22	22	December 30, 1926	5	.....	....	July 8, 1927	.3	Alive October 7, 1927
19	16	Control		July 8, 1927	.2	... .	.....	Died August 14, 1927†
37	30	Control		July 8, 1927	.2	....	....	Alive October 7, 1927
38	20	Control		July 8, 1927	.2	... .	.....	Alive October 7, 1927
39	20	Control		.....	.....	July 8, 1927	.3	Alive October 7, 1927
45	24	Control		.....	....	July 8, 1927	.3	Died October 2, 1927†

*Brain of rabid dog placed in glycerin May 24, 1927.
†Negative for rabies as determined by microscopical examination of brain and intracranial inoculation of rabbits.

TABLE 3.—Rabies Immunization Tests 6 Months After Vaccination

GROUP C

Dog	Weight	Vaccinated	Amount injected	Street virus* injected intraocularly	Amount	Street virus* injected traumatically	Amount	Results
No.	*Lb.*	*Date*	*cc.*	*Date*	*cc.*	*Date*	*cc.*	
23	30	December 30, 1926	5	July 8, 1927	0.2			Alive October 7, 1927
24	13	December 30, 1926	5	July 8, 1927	.2			Alive October 7, 1927
25	21	December 30, 1926	5	July 8, 1927	.2			Alive October 7, 1927
26	20	December 30, 1926	5	July 8, 1927	.2			Alive October 7, 1927
27	32	December 30, 1926	5	July 8, 1927	.2			Alive October 7, 1927
28	29	December 30, 1926	5			July 8, 1927	0.3	Alive October 7, 1927
30	19	December 30, 1926	5			July 8, 1927	.3	Alive October 7, 1927
32	31	December 30, 1926	5			July 8, 1927	.3	Alive October 7, 1927
33	20	December 30, 1926	5			July 8, 1927	.3	Alive October 7, 1927
29	20	Control		July 8, 1927	.2			Died July 27, 1927 R.†
40	17	Control		July 8, 1927	.2			Died July 28, 1927 R.
41	15	Control		July 8, 1927	.2			Died July 26, 1927 R.
31	22	Control				July 8, 1927	.3	Alive October 7, 1927
42	16	Control				July 8, 1927	.3	Alive October 7, 1927

*Brain of rabid dog placed in glycerin May 13, 1927.
†R Died of rabies—confirmed by demonstration of Negri bodies.

The results obtained in the control dogs suggest that there was a variation in the virulence of the three viruses used in these tests. In Groups A and C the viruses employed were virulent when judged by the fatal infections they produced and the uniformity of this virulence is indicated by the period of incubation. The results obtained in Group B would tend to show that this virus was avirulent. Evidently the variation in virulence of the viruses was not the result of the different periods of time during which the brains were held in storage, as the B virus, apparently the least virulent, had been placed in glycerin eleven days later than viruses A and C. All storage conditions of the viruses were the same.

All vaccinated dogs in Group A died and Negri bodies were demonstrated in each, with the exception of dog No. 2. Negri bodies were not demonstrated in the brain of dog No. 2, nor did rabbits develop rabies following an intracranial inoculation of the brain substance of this dog. The results obtained in Group A would indicate that the vaccine did not protect the dogs against an injection of this strain of rabic virus six months following vaccination.

The dogs in Group B with the exception of two controls remained alive. Negri bodies were not found in the brains of these control dogs, nor did rabies develop in rabbits inoculated intracranially with the brain substance. Since none of the controls developed rabies, conclusions could not be drawn as to the immunity of the vaccinated dogs in this group.

All vaccinated dogs in Group C remained alive, while the three controls receiving intraocular injections died of rabies. The results of this test indicate that an immunity against rabies existed in the vaccinated dogs.

SUMMARY

In this experiment no dog developed rabies following the traumatic injection of street virus.

Rabies did not develop in vaccinated or control dogs following an intraocular injection of one of the strains of virus.

The vaccinated dogs were protected against an intraocular injection of one strain of rabic street virus, while against another strain of street virus there was no protection.

EXPERIMENT II

The results obtained in the first experiment indicated a difference in strains of rabic street virus and this stimulated further inquiry. Dogs from the former experiment were used in

Experiment II. Since none of the dogs injected traumatically in the former experiment developed rabies, they were thought to be suitable for determining the virulence of other strains of rabic street virus. It was also of interest to know whether or not the dogs that had withstood an intraocular injection of virus would prove susceptible to another strain of street virus.

For this test there were available from the first experiment, twenty vaccinated and eight control dogs. Ten of the vaccinated dogs had received intraocular injections and the other ten traumatic injections of rabic street virus. Of the eight controls two had been injected intraocularly and six traumatically.

Assuming that the injection of virus had not influenced their immunity, the vaccinated dogs afforded an opportunity of testing susceptibility nine months after vaccination. The control dogs also could be subjected to infection with other strains of rabic street virus.

PLAN OF EXPERIMENT

The dogs were divided into two groups, "D" and "E". In each group were five vaccinated dogs that had been inoculated intraocularly and five traumatically. Group D also contained five former controls, one of which had been injected with virus intraocularly and four traumatically, while Group E had three former controls, one injected intraocularly and two traumatically.

Eight additional dogs obtained from the pound were used as controls in this experiment, five of them were added to Group D and three to Group E. These dogs were held under observation for thirty days before being used and all were kept under conditions similar to those in the former experiment.

The inoculation viruses for this experiment consisted of suspensions of the brain of two rabid dogs. These cases were obtained from two separate outbreaks of rabies. One brain had been kept in glycerin for six and the other eight days prior to injection. The brain suspensions were prepared as described in the first experiment, except that one part of brain was used to ten of the diluent. Each dog received an intraocular injection October 7, 1927 of 0.2 cc. of this suspension.

DISCUSSION

A comparison of the results obtained following virus inoculations of the former traumatically injected controls and new controls, gives some evidence regarding the degree of immunity produced in the dogs by the former virus injections.

TABLE 4.—Rabies Immunization Tests 9 Months After Vaccination

GROUP D

Dog	Vaccinated	Amount injected	Street virus* injected intraocularly	Street virus* injected traumatically	Street virus† injected intraocularly	Amount	Results
No.	*Date*	*cc.*	*Date*	*Date*	*Date*	*cc.*	
10	December 30, 1926	5	....	"A" July 8, 1927	October 7, 1927	0.2	Alive January 9, 1928
12	December 30, 1926	5	....	"A" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
13	December 30, 1926	5	"B" July 8, 1927	....	October 7, 1927	.2	Alive January 9, 1928
14	December 30, 1926	5	"B" July 8, 1927	.. ..	October 7, 1927	.2	Alive January 9, 1928
18	December 30, 1926	5	....	"B" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
21	December 30, 1926	5	....	"B" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
23	December 30, 1926	5	"C" July 8, 1927	.....	October 7, 1927	.2	Alive January 9, 1928
24	December 30, 1926	5	"C" July 8, 1927	. ...	October 7, 1927	.2	Alive January 9, 1928
25	December 30, 1926	5	"C" July 8, 1927	.. ..	October 7, 1927	.2	Alive January 9, 1928
30	December 30, 1926	5	.....	"C" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
35	Former control		.. . .	"A" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
43	Former control		....	"A" July 8, 1927	October 7, 1927	.2	Died October 28, 1927 R.‡
37	Former control		"B" July 8, 1927	....	October 7, 1927	.2	Alive January 9, 1928
39	Former control		....	"B" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
31	Former control		....	"C" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
46			Control		October 7, 1927	.2	Alive January 9, 1928
47			Control		October 7, 1927	.2	Died November 3, 1927 R.
48			Control		October 7, 1927	.2	Alive January 9, 1928
52			Control		October 7, 1927	.2	Alive January 9, 1928
53			Control		October 7, 1927	.2	Died November 3, 1927 R.

*Virus A, B, and C used in experiment No. 1.
†Brain of rabid dog placed in glycerin September 29, 1927
‡R Died of rabies—confirmed by demonstration of Negri bodies.

TABLE 5.—Rabies Immunization Tests 9 Months After Vaccination

GROUP E

Dog	Vaccinated	Amount injected	Street virus* injected intraocularly	Street virus* injected traumatically	Street virus† injected intraocularly	Amount	Results
No.	*Date*	*cc.*	*Date*	*Date*	*Date*	*cc.*	
8	December 30, 1926	5	.....	"A" July 8, 1927	October 7, 1927	0.2	Alive January 9, 1928
9	December 30, 1926	5	.....	"A" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
15	December 30, 1926	5	"B" July 8, 1927	.....	October 7, 1927	.2	Alive January 9, 1928
16	December 30, 1926	5	"B" July 8, 1927	....	October 7, 1927	.2	Alive January 9, 1928
17	December 30, 1926	5	"B" July 8, 1927	.....	October 7, 1927	.2	Alive January 9, 1928
20	December 30, 1926	5	.....	"B" July 8, 1927	October 7, 1927	.2	Died December 19, 1927 R.‡
26	December 30, 1926	5	"C" July 8, 1927	.....	October 7, 1927	.2	Alive January 9, 1928
27	December 30, 1926	5	"C" July 8, 1927	....	October 7, 1927	.2	Alive January 9, 1928
32	December 30, 1926	5	.....	"C" July 8, 1927	October 7, 1927	.2	Died October 30, 1927 R.
33	December 30, 1926	5	....	"C" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
36	Former control		.....	"A" July 8, 1927	October 7, 1927	.2	Died November 1, 1927 R.
38	Former control		"B" July 8, 1928	.....	October 7, 1927	.2	Killed November 29, 1927§
42	Former control		.....	"C" July 8, 1927	October 7, 1927	.2	Alive January 9, 1928
50	Control				October 7, 1927	.2	Died November 10, 1927 R.
51	Control				October 7, 1927	.2	Died October 25, 1927 R.
55	Control				October 7, 1927	.2	Died November 2, 1927 R.

*Virus A, B, and C used in experiment No. 1.
†Brain of rabid dog placed in glycerin October 1, 1927.
‡R Died of rabies—confirmed by demonstration of Negri bodies.
§Killed on account of mange.

In Group D one of the four former controls died of rabies; while of the five new controls two died of this disease. In Group E one of two former controls and the three new controls died of rabies.

While the number of animals in these groups is too small to allow the formation of definite conclusions, the results suggest that little immunity was conferred by the traumatic injections of street virus in the former test. None of the dogs injected intraocularly in the first experiment developed rabies as a result of the second injection. Whether this resistance was due to a natural immunity or resulted from previous inoculations, could not be determined.

In Group D the ten vaccinated dogs remained alive, while two of the five controls died of rabies. In Group E two of the vaccinated dogs and the three controls died of rabies. The experiment was terminated three months after the virus was inoculated. Judging from the mortality in the controls it would appear that the virus used in Group E was more virulent than that of Group D. Similarly the vaccinated dogs showed less resistance to street virus "E" than to "D".

It is of interest to note that all dogs, either vaccinated or controls, which had withstood one intraocular injection of rabic virus, remained resistant to the injection of other street virus administered three months later.

SUMMARY

Dogs inoculated traumatically with rabic street virus did not develop an appreciable immunity, as judged by the results obtained following an intraocular injection of street virus three months later.

In the group of dogs that had been vaccinated nine months, the protection was relatively high when compared with the controls.

CONCLUSIONS

These experiments indicate that:

Street virus of rabies from different sources varies in virulence.

The single injection of a vaccine, prepared according to a modification of the method suggested by Umeno and Doi, protected dogs against virus from two sources; towards another virus a high degree of immunity was shown; while no protection was evident to a virus from a still different source.

Vaccinated dogs were resistant to infection with some strains of rabic street virus six and nine months following vaccination.

The single injection method of vaccination of dogs against rabies is commendable from a practical standpoint; however, vaccination cannot be depended upon to eradicate rabies until a method is devised that will protect dogs against all strains of rabic street virus.

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