Experimental Use in Dogs of Rabies Vaccine Prepared in Embryonated Duck Eggs

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The preparation of rabies vaccine in embryonated duck eggs has already been described (1, 2, 3). Its use in man has been documented in several papers (4, 5, 6).

Briefly this vaccine comprises fixed rabies virus of first generation duck embryo passage, and this is put up in freeze dried form to be rehydrated with sterile water at the time of use. Assay of potency is accomplished by use of N. I. H. mouse immunization and challenge methods. The need for purification to remove factors present in brain tissue vaccine responsible for neuroparalytic accidents is practically nil in the case of duck embryo vaccine which virtually lacks such properties (7).

In addition to efficient immunization of mice as done in the N. I. H. assay tests, the inactivated duck embryo rabies vaccine has been found capable of producing virus neutralizing antibody in rabbits, monkeys, and human beings. Vaccinated rabbits and guinea pigs were found to develop immunity to street virus (8).

Materials and Methods

The results reported here concern an experiment with active duck embryo rabies vaccine in dogs. Two of us (H. M. P. and C. G. C.) furnished duck embryo vaccine which had passed through the official N. I. H. mouse assay tests twice. J. MacF. and F. O. G. administered this to dogs subcutaneously and provided serum bleedings from these dogs under code numbers for serum-virus neutralization tests by H. M. P. and C. G. C. Commercial veterinary phenolyzed vaccine furnished by J. MacF. and F. O. G. was used for comparison. Details of the work with dogs by J. MacF. and F. O. G. were not known to H. M. P. and C. G. C. at the time. Certain groups of sera were designated for test on any one day so that pre and post immune sera from the same animals would be tested together.

Serum-virus neutralization tests were conducted with serum samples inactivated at 56°C for 30 minutes. Doubling dilutions of serum, i.e. 1:2, 1:4, 1:8, 1:16, etc. were prepared in saline. One volume of such serum dilutions was added to one volume of properly diluted virus, and the mixture incubated 1 hour at 37° C. Final serum dilutions thus became 1:4, 1:8, 1:16, 1:32, etc. Groups of 6 mice were then injected intracerebrally with 0.03 cc of the various mixtures. The virus in the mixture as noted above was diluted so each mouse got 100 LD_{50} of fixed virus as proven by 40 virus controls used each day. Final readings of mice dead and mice surviving were made 14 days later.

From these data, exact titer of each serum was computed by the methods of Reed and Muench (9), and titers are expressed as the reciprocal of the final dilution of serum which protected half the mice, i.e. 1:4 and 1:8 are expressed as 4 and 8, etc.

Results

When these tests were completed and all titers had emerged, exchange of information revealed that 28 dogs were given single doses of 3 cc of

duck embryo vaccine following a normal bleeding, while 15 dogs were given single doses of 5 cc of commercial brain origin veterinary vaccine following a similar normal bleeding. Thirty days later single post-immunization bleedings were made from all dogs.

A third vaccine was originally included in these tests. This was a commercial vaccine using a 3 cc dose however the results of this were so far below expectations they are not being reported.

TABLE I

Dogs Receiving Duck Embryo Vaccine (single dose of 3 cc)						Dogs Receiving Commercial Vaccine (single dose of 5 cc)		
Dog Number	Initial Titer	30 Day Titer	Dog Number	Initial Titer	30 Day Titer	Dog Number	Initial Titer	30 Day Titer
4704	0	5.7	*4720	19.4	84.6	4734	0	45.4
4705	0	4.5	4721	0	16	4735	0	0
4706	0	21.7	4723	0	12.6	4736	0	19
4707	0	13.9	4724	0	3.5	4737	0	91.6
4708	0	0	4725	0	4	*4740	4	42
4709	0	7.1	4726	0	18	4742	0	17.4
4710	0	49.4	4727	0	14.2	4744	0	45.4
4711	0	0	4728	0	6.5	4745	0	0
4712	0	48.8	4729	0	6	4746	0	9
4713	0	7.3	4731	0	4	4747	0	42
4714	0	16	*4732	4	4	4748	0	28.4
4715	0	0	4733	0	147	4750	0	48
4716	0	14.4	4738	0	0	4751	0	19
4718	0	21	4749	0	0	4752	0	4
						4753	0	0

* Dogs 4720, 4732, and 4740 exhibited an antibody titer in the initial (pre-immunizations) bleeding. Vaccination increased the titers of 4720 and 4740 but not of 4732. Dog 4732 contracted pneumonia during the 30 day period and this may have inhibited a normal response.

Table I shows a list of all dogs used in the experiment, their normal rabies virus neutralizing titer, and their post immunization titer 30 days following the single injection of vaccine.

Inspection of Table I shows two dogs in the duck embryo vaccine group (numbers 4720 and 4732) and one dog in the brain vaccine group (4740) had antibody to begin with, that is in the initial or pre-immunization bleeding. Two of these, 4720 and 4740, increased in titer while one dog, 4732, did not. This latter dog developed pneumonia during the 30 days period and this infection might have suppressed a normal response to vaccine. In order to figure response to vaccine later in this report, we shall omit the three dogs showing virus neutralizing antibody in the initial bleeding.

It is further evident from Table I that five dogs of 28 in the duck embryo vaccine group, and three dogs of 15 in the brain vaccine group showed no antibody response at 30 days after vaccination. Our tests, and the lowest thresholds tested, do not preclude the possible presence of tracts of antibody if at the strongest serum dilution used, namely 1:4,

our results are negative. In other words, more sensitive tests might have revealed weaker antibody in the dogs whose response we regard as negative.

TABLE 2

Vaccine Used	Number Dogs in Group	Number of Dogs Having Virus-Neutralizing Titers of: 0	4*	8	16	32	64	128
Duck Embryo 3cc	26	5	5	4	9	0	2	1
Commercial 5cc	14	3	1	1	3	4	2	0

* Titers expressed as in Table I. Each dog assigned to "nearest dilution" group on basis of its final computed titer.

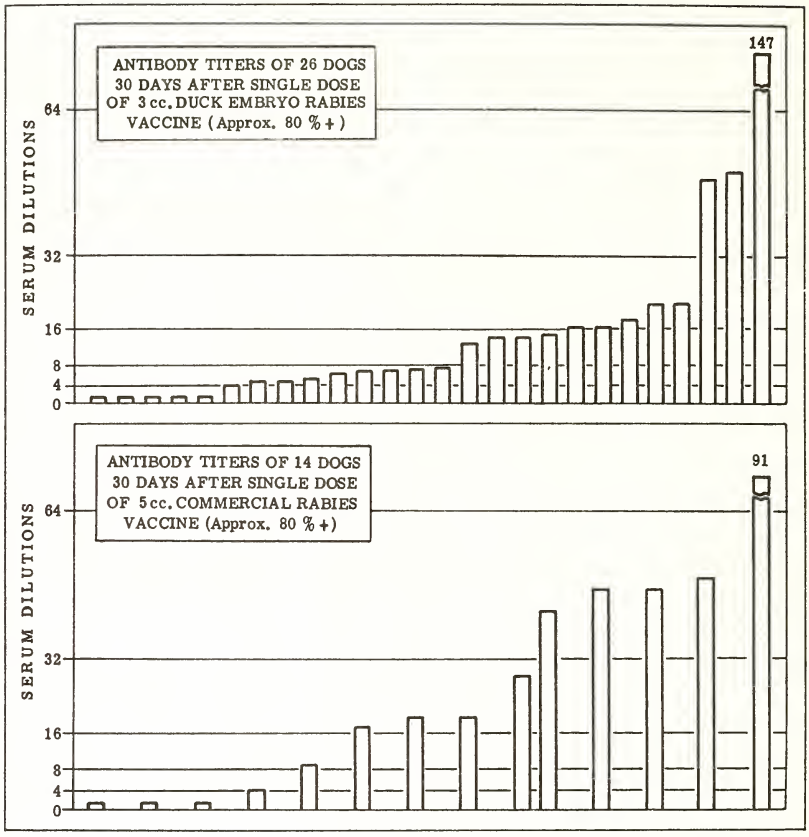
In Table 2 we have tabulated all dogs used in increasing order of final potency with the exception of the three animals mentioned above which had antibody to begin with. In the groupings shown in Table 2, individual animals were assigned to an antibody titer group to which their computed titers were nearest.

It appears from Table 2 that approximately 80 percent of each group of dogs developed antibody 30 days after an injection of either vaccine. Twenty-one of twenty-six dogs on duck embryo vaccine, and eleven of fourteen dogs on commercial vaccine developed antibody. Roughly the success of each vaccine in this respect turned out to be about the same as based on the limited number of animals used. However inspection of Table 2 indicates the antibody titers in the former group are not as high as those in the latter group. Obviously it would take more animals to settle this point, however the indications of this difference are in line with the use as mentioned above of 3 cc of duck embryo vaccine in comparison with 5 cc of brain vaccine. Since both of these vaccines originate from fixed virus it might have been more advantageous to have compared these two vaccines on an equal-volume-of-dose basis.

A bar graph chart I has been made on an arithmetical basis of virus neutralizing titers of the two groups of dogs. It appears that although there is little difference in the relative numbers of dogs successfully immunized by the two vaccines (roughly 4 out of 5 dogs developing a demonstrable serum titer at 30 days), the height of antibody titers is greater in some of the brain vaccine group than in the duck embryo vaccine group. Since the duck embryo vaccine group of dogs got 3 cc of vaccine per dog and the brain vaccine group of dogs got 5 cc of vaccine per dog, it would appear reasonable to expect a 5 cc dose of duck embryo vaccine to equal a similar dose of brain vaccine as regards antibody response in dogs.

Conclusions

1. A single dose of 3 cc of duck embryo rabies vaccine given to dogs, with no antirabies antibody in their serum to begin with, results in demonstrable antibody titers in about 80% of dogs in 30 days.



2. A single dose of 5 cc of commercial rabies vaccine of brain origin under the same conditions produces antibody also in about 80% of dogs.
3. So called long incubation antibody tests might have increased these percentages.
4. The brain vaccine (5 cc) dogs have somewhat higher titers than the duck embryo vaccine (3 cc) dogs. This might be expected in view of the differing doses of vaccine.
5. These showings are compatible with the probability that equal doses of these two vaccines would be quite comparable immunologically, although equal doses were not tested in this study.
6. Although the duck embryo rabies vaccine used in this experiment comprised live virus, no ill effects were produced in dogs. There was no particular merit in this vaccine over a similar inactivated vaccine which we reported effective in 34 of 43 dogs two years ago.
7. The dogs used in this study were for general pharmacological use and hence were not challenged with street virus.

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