A DOG TEST FOR MEASURING THE IMMUNIZING POTENCY OF ANTIRABIES VACCINES

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Plate 35

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In a previous paper we described a mouse test for determining quantitatively the immunizing potency of antirabies vaccines (1). That test showed commercial phenolized vaccines for the most part to be incapable of rendering mice immune to a subsequent injection of rabies virus when employed in doses comparable to those for dogs or man with respect to body weight. Commercial chloroformized vaccines from two firms, on the other hand, showed definite immunizing capacity provided two to five times the comparative dose was given intraperitoneally rather than subcutaneously.

These findings have now been checked in dogs by determining whether canine vaccines which fail to immunize mice, likewise fail to immunize dogs, and conversely, whether vaccines plus procedures which successfully immunize mice, will likewise immunize dogs. The results of these tests thus far parallel those of the mouse tests (2, 3) and are described here in detail.

Technique for Testing Single Injection Antirabies Vaccines in Dogs

According to reports of previous workers, rabies vaccines prove capable of inducing a well defined immunity in animals only if they are administered prophylactically, that is, prior to a test injection of virus (5). Moreover, no method of testing thus far reported, save the one in mice (1), has yielded quantitative data on the amount of immunity obtained per unit of vaccine. The following test in dogs has been developed after the pattern of the mouse test to provide a quantitative result under conditions as nearly natural as possible.

Beagle dogs are used because of their small size and quiet disposition. Animals of the same age, that is, 4 to 6 months, are chosen because age factors are known to influence markedly the susceptibility and immunizability of mice to rabies (4), and they may play an equally important rôle with dogs. The young animals are brought to the premises usually in batches of about thirty-five, placed in quarantine rooms, wormed,

and given 10 to 15 cc. of distemper immune serum, followed 3 days later by one injection of non-virulent distemper vaccine.

About 7 days after their arrival, the animals are injected with the antirabies vaccines according to the requirements of the experiment. Five to fifteen animals remain unvaccinated, as controls.

3 weeks later, all are tested for immunity by an injection into the neck muscles of each side with 0.25 cc. of virus properly diluted. Dilutions are employed which are calculated to contain approximately 1, 10, or 100 lethal doses of virus (see Table I). The injected animals are transferred to single cages and observed 2 to 5 months. Those found prostrate or dead are autopsied, their brains removed, and a portion of the latter is inoculated intracerebrally into mice for identification of the virus. Immunity is measured in terms of number of lethal doses resisted by at least 50 per cent of the dogs.

The test virus, strain 15811, was obtained in 1937 from a rabid street dog. The virus-containing dog brain was passed intracerebrally to Swiss mice. When the animals became prostrate, the infected mouse brains were removed and stored in glycerin. Prior to each experiment, the glycerinated mouse brain virus is passed once intracerebrally through mice and the brains from the resulting prostrate animals are used to inoculate the test dogs. When the non-vaccinated dogs become prostrate, their brains in turn are passed intracerebrally through mice. The brains of these mice are removed when the animals become prostrate and stored in glycerin to serve as virus for the next dog experiment. Thus far, the infected mouse brains have continued to show large and numerous Negri bodies (Fig. 1).

Intramuscular Virulence for Dogs of Rabies Virus 15811

Experiments with various routes of injecting test virus into dogs resulted in the selection of the neck muscles as most natural and at the same time yielding the most measurable titration results. English investigators preferred this route as a result of 20 years' study of rabies vaccines (5). In our experience, titrations by the intracerebral route proved simple and gave quantitative results, but they were so severe that vaccinated dogs rarely withstood more than one lethal dose (6). Titrations by the lingual route likewise gave results capable of reproduction in our hands, provided the vaccinated dogs were lightly anesthetized and injected with virus into the same portion of the tongue and at the same depth. Titrations by the masseter muscle route gave less regular results in duplicate and repeated tests. Finally, titrations by the gastrocnemius muscle route provided data too variable for quantitative studies.

The results of titrations of rabies virus, No. 15811, into the neck muscles of beagle dogs, as described above, are shown in Tables I and II.

In Table I, the results of each test are set forth under the headings of dilution of virus injected, in terms of survival time in days of each fatal case, plus the number of survivors. Taken together, they reveal that in four tests in which the 1:50 dilution was given, a total of seventeen of

TABLE I

Neck Muscle Titrations of Dog Passage Virus, 15811, in 5 Months Old Beagle Dogs

Test	Fate of dogs injected with 0.25 cc. of virus into neck muscles of right and left sides in dilutions								
	1:50	1:100	1:200	1:400	1:500	1:2,500	1:4,000	1:10,000	1:40,000
P	15*, 16, 26								
1	14, 17, 17, 18, 22, 23				12, 14, 18				
2	11, 11, 11, 13								
3		12, 13, 14, 16			14, 18, S, S	17, 17, 17, 18			
4	13, 15, 15, 17				12, 14, 15, 27, 52			15, 18, 18, 23, 31	
5		12, 15, 17, 18, 18							
6			12, 13, 14, 23, 34						
7			14, 15, 18, 18, 21, 22				16, 23, 39, S, S		22, 42, S, S
8				15, 18, S, S			16, 16, 16, 19, 19		22, S, S, S
10				12, 19, S			15, 16, 18, 30, S		39, 42, S, S
11			14, 16, S, S, S				18, 20, 22, 22, 33, S, S, S, S, S		S, S, S, S, S
Totals..	17 of 17 = 100%	9 of 9 = 100%	13 of 16 = 81%	4 of 7 = 57%	10 of 12 = 83%	4 of 4 = 100%	17 of 25 = 68%	5 of 5 = 100%	5 of 17 = 29%
Combined totals	1:50 to 1:200 inclusive, 39 of 42 = 93%			1:400 to 1:10,000 inclusive, 40 of 53 = 75%					5 of 17 = 29%

* Day of death from rabies following injection.

S = remained well.

seventeen dogs succumbed (100 per cent); in two tests in which the 1:100 dilution was given, nine of nine (100 per cent), and in three tests in which the 1:200 dilution was given, a total of thirteen of sixteen dogs (81 per cent) succumbed. Again, in two tests in which the 1:400 dilution was given, four of seven (57 per cent) succumbed; in three in which the 1:500 dilution was given, ten of twelve (83 per cent); in one test with the 1:2,500 dilution, four of four (100 per cent); in four tests with the 1:4,000 dilution, seventeen of twenty-five (68 per cent), and in one test with the 1:10,000 dilution, five of five succumbed (100 per cent). Finally, in four tests in which the 1:40,000 dilution was given, five of seventeen (29 per cent) succumbed. Combining these figures again into groups in which the mortality was consistently: (*a*) close to 100 per cent; (*b*) less than 100 per cent but

TABLE II

Duration of Life of Dogs Following Injection of Dog Passage Rabies Virus, 15811, into the Neck Muscles of Young Beagle Dogs

Duration of life of injected dogs	Number and per cent of dogs succumbing at stated intervals following injection of virus in dilutions					
	1:50 to 1:200		1:400 to 1:10,000		1:40,000	
	No.	*per cent*	*No.*	*per cent*	*No.*	*per cent*
11 to 19 days	32/42	76	29/53	55	0/17	0
20 to 29 "	7/42	17	6/53	11	2/17	12
30 to 60 "	0	0	5/53	9	3/17	18
Survived 2 mos.	3/42	7	13/53	25	12/17	70

greater than 50 per cent, and (*c*) less than 50 per cent, we note that (*a*) dilutions of 1:50 to 1:200 inclusive resulted in a mortality of thirty-nine of forty-two tested dogs (93 per cent); (*b*) that dilutions of 1:400 to 1:10,000 produced a mortality of forty of fifty-three dogs (75 per cent), and (*c*) that dilutions of 1:40,000 brought about a mortality of five of seventeen tested dogs (29 per cent). From these figures we have taken dilution 1:200 as the least dose fatal to practically 100 per cent of test animals and dilution 1:10,000 as the least dose fatal to more than 50 per cent but less than 100 per cent.

These end points are in keeping with figures obtained by grouping the durations of life of the dogs shown in Table I.

Table II shows that of forty-two dogs given the 1:50 to 1:200 dilutions of virus, 76 per cent died in 11 to 19 days, whereas of fifty-three given the 1:400 to 1:10,000 dilutions, 55 per cent succumbed and of seventeen given the 1:40,000 dilution, none succumbed within this 11 to 19 day period.

The percentages succumbing within 20 to 60 days were small (0 to 18) in all groups. Finally, of seventeen dogs given the 1:40,000 dilution, none died within the 11 to 19 day period, 30 per cent in the 20 to 60 day period, and 70 per cent remained well. In short, there is an increase in survival time as well as survival percentages at the 1:200, 1:10,000, and 1:40,000 dilution levels of virus.

Immunizing Potency of Commercial Canine Antirabies Vaccines

The above approximations of minimum lethal doses of virus were useful in evaluating our tests on dogs with commercial canine vaccines. At the outset, animals following vaccination were tested against two to four doses of virus fatal to nearly 100 per cent of controls—1:200 to 1:50 dilutions. Subsequently, however, in view of the failure of vaccinated animals to withstand this dose, they were tested against a much smaller amount of virus, namely, two to twenty minimum doses, fatal to more than 50 per cent but less than 100 per cent of controls—the 1:4,000 to 1:400 dilutions. These results likewise proved negative, as illustrated by the following series of protocols.

Experiment 1 (*Test 5*).—5 cc. of phenolized vaccine No. 4 were injected subcutaneously into each of five dogs; 5 cc. of No. 5 phenolized vaccine into five dogs; 5 cc. of No. 8 phenolized and 5 cc. of No. 9 each subcutaneously into each of five dogs respectively. Four dogs received 5 cc. of No. 1 chloroformized vaccine subcutaneously, and five dogs remained unvaccinated as controls. 3 weeks later each received 0.25 cc. of the 1:100 dilution of No. 15811 virus into the neck muscles of each side.

All five controls died of rabies on the 12th to 18th days following injection of test virus (100 per cent) (Table III). The five dogs receiving the No. 4 vaccine likewise died on the 9th to 26th days (100 per cent), and the five dogs receiving the No. 5 vaccine on the 12th to 18th days (100 per cent). Four of the five receiving the No. 8 vaccine died on the 12th to 19th days (80 per cent), and one survived; similarly, four of five receiving the No. 9 vaccine died on the 12th to 21st days (80 per cent), and one survived. In contrast to this, only one of the four dogs receiving the chloroformized vaccine No. 1 succumbed (25 per cent).

Table III shows that none of the phenolized vaccines protected dogs to the slightest degree against the 1:100 dilution of test virus, whereas the chloroformized vaccine afforded significant protection. The 1:100 dilution is regarded as two minimum doses fatal to 100 per cent of controls.

Experiment 2 (*Test 7*).—Six dogs, lot A, remained unvaccinated. Three dogs, lot B, each received 5 cc. of phenolized vaccine No. 3 subcutaneously. Six dogs, lot C, each received 5 cc. of chloroformized vaccine No. 3 subcutaneously. Five dogs, lot D, remained unvaccinated. Four dogs, lot E, received 5 cc. of phenolized vaccine No. 3 subcutaneously, and four dogs, lot F, remained unvaccinated. 3 weeks later, lots A, B, and C

each received 0.25 cc. of test virus diluted 1:200 into the neck muscles of each side. Lots D and E received the same dose of the 1:4,000 dilution, and lot F, the 1:40,000 dilution.

All six controls in lot A, receiving the 1:200 dilution, died of rabies on the 14th to 22nd days following injection (100 per cent) (Table IV). Similarly, two of the three in lot B receiving the phenolized No. 3 died on the 12th and 16th days (66 per cent), and five of six in lot C receiving the chloroformized vaccine died on the 13th to 23rd days (84 per cent). Three of five controls in lot D receiving the 1:4,000 dilution died on the 16th, 23rd, and 39th days respectively (60 per cent), and two of four in lot E receiving the phenolized No. 3 vaccine died on the 15th and 18th days (50 per cent). Finally, of the four control dogs in lot F given the 1:40,000 dilution, two died on the 22nd and 42nd days (50 per cent).

TABLE III

Immunizing Effects of Canine Antirabies Vaccines on Beagle Dogs

Experiment 1, Test 5

Treatment of dogs	Fate of dogs inoculated into the neck muscles (right and left) with 0.25 cc. of dog passage virus, 15811, diluted 1:100		
	Day of death following inoculation	No. dead/ No. injected	Dead
			per cent
A. No vaccine	12, 15, 17, 18, 18	5/5	100
B. Vaccine 4: Phenol, 5 cc. subc.	9, 10, 15, 18, 26	5/5	100
C. " 5: " " " "	12, 15, 16, 17, 18	5/5	100
D. " 8: " " " "	12, 13, 14, 19, S	4/5	80
E. " 9: " " " "	12, 13, 14, 21, S	4/5	80
F. " 1: Chloroform, 5 cc. subc.	13, S, S, S	1/4	25

S = animal remained well following injection. Survivors discarded after 60 days.

In this, as in the above experiment, there was no significant difference in the mortality of unvaccinated dogs given the 1:200 test dose (100 per cent) and in vaccinated dogs similarly tested (66 per cent and 84 per cent). Moreover, when the test dose was reduced to 1:4,000, a point close to the minimum dose fatal to 50 to 100 per cent, there was still no significant protecting effect of the vaccine,—controls 60 per cent as compared to vaccinated dogs 50 per cent.

Experiment 3 (Test 8).—Four dogs, lot A, remained unvaccinated. Each of three dogs, lot B, received 5 cc. of phenolized vaccine No. 9 subcutaneously, and four dogs, lot C, each received 5 cc. of chloroformized vaccine No. 1 subcutaneously. Five dogs, lot D, remained unvaccinated; three dogs, lot E, each received 5 cc. of phenolized vaccine No. 9 subcutaneously; four dogs, lot F, each received 5 cc. of phenolized vaccine No. 4 subcutaneously; and five dogs, lot G, each received 5 cc. of chloroformized vaccine

No. 1 subcutaneously. The final lot, H, remained unvaccinated. 3 weeks later each dog in lots A, B, and C received 0.25 cc. of test virus diluted 1:400 into the neck muscles of each side, each dog in lots D, E, F, and G, the same amount of virus diluted 1:4,000, and each dog in lot H, the same dose diluted 1:40,000.

Two of the four unvaccinated dogs of lot A died of rabies on the 15th and 18th days respectively (50 per cent). Two of the three vaccinated dogs in lot B died of rabies on the 13th and 14th days (66 per cent), and four of the four vaccinated dogs in lot C on the 15th, 17th, 19th, and 22nd days (100 per cent). Five of five unvaccinated dogs in lot D given the smaller dose of virus died on the 16th, 16th, 16th, 19th, and 19th days respectively (100 per cent). One of three vaccinated dogs in lot E died of rabies on the 19th day (33 per cent), three of four vaccinated dogs in lot F likewise succumbed on the 21st day (75 per cent), and two of five vaccinated dogs in lot G died on the 15th and

TABLE IV

Immunizing Effects of Canine Antirabies Vaccines on Beagle Dogs

Experiment 2, Test 7

Treatment of dogs	Dilution of test virus	Fate of dogs inoculated into the neck muscles (right and left) with 0.25 cc. of dog passage virus, 15811		
		Day of death following inoculation	No. dead/ No. injected	Dead
				per cent
A. No vaccine	1:200	14, 15, 18, 18, 21, 22	6/6	100
B. Vaccine 3: Phenol, 5 cc. subc.	"	12, 16, S	2/3	66
C. " 3: Chloroform, 5 cc. subc.	"	13, 16, 17, 21, 23, S	5/6	84
D. No vaccine	1:4,000	16, 23, 39, S, S	3/5	60
E. Vaccine 3: Phenol, 5 cc. subc.	"	15, 18, S, S	2/4	50
F. No vaccine	1:40,000	22, 42, S, S	2/4	50

S = animal remained well following injection. Survivors discarded after 47 days.

22nd days (40 per cent). Finally, one of four unvaccinated dogs in lot H succumbed to the experimental injection on the 22nd day (25 per cent).

This experiment shows no protective effect of any vaccine. Lots A, B, and C received a dilution of 1:400, calculated as twenty minimum doses fatal to 50 per cent but less than 100 per cent. In this group, 50 per cent of the controls, A, died from the test injection, as compared with 66 and 100 per cent of the vaccinated B and C lots respectively. Again, lots D, E, F, and G received a still smaller test dose, 1:4,000, which is not more than two minimum doses fatal to 51 to 99 per cent. 100 per cent of the controls died, as compared to 33, 75, and 40 per cent of the vaccinated dogs in lots E, F, and G respectively. This 50 per cent or more mortality on the part of the vaccinated does not differ significantly from the average

figure for controls given this dilution (72 per cent), or indeed from the somewhat irregular figure (100 per cent) in this test.

Experiment 4 (Test 10).—Three dogs, lot A, five in lot B, and four in lot H remained unvaccinated. Each of five dogs in lot C received 5 cc. of phenolized vaccine No. 10 subcutaneously, and each of five in lot D, 5 cc. of chloroformized vaccine No. 1 subcutaneously. 3 weeks later, each dog of lot A received 0.25 cc. of virus 15811 diluted 1:400 into the neck muscles of each side, each of lots B, C, and D the same volume of virus diluted 1:4,000, and each of lot H, 1:40,000.

Two of the three control dogs, lot A, given the 1:400 dilution of virus died on the 12th and 19th days (66 per cent). Four of five controls, lot B, given the 1:4,000 dilution

TABLE V

Immunizing Effects of Canine Antirabies Vaccines on Beagle Dogs

Experiment 3, Test 8

Treatment of dogs	Dilution of test virus	Fate of dogs inoculated into the neck muscles (right and left) with 0.25 cc. of dog passage virus, 15811		
		Day of death following inoculation	No. dead/ No. injected	Dead
				per cent
A. No vaccine	1:400	15, 18, S, S	2/4	50
B. Vaccine 9: Phenol, 5 cc. subc.	"	13, 14, S	2/3	66
C. " 1: Chloroform, 5 cc. subc.	"	15, 17, 19, 22	4/4	100
D. No vaccine	1:4,000	16, 16, 16, 19, 19	5/5	100
E. Vaccine 9: Phenol, 5 cc. subc.	"	19, S, S	1/3	33
F. " 4: " " " "	"	21, 21, 21, S	3/4	75
G. " 1: Chloroform, 5 cc. subc.	"	15, 22, S, S, S	2/5	40
H. No vaccine	1:40,000	22, S, S, S	1/4	25

S = animal remained well following injection. Survivors discarded after 41 days.

of virus died on the 15th, 16th, 18th, and 30th days (80 per cent); four of five vaccinated dogs, lot C, given the same dilution of test virus, died on the 14th, 16th, 17th, and 43rd days (80 per cent), and two of the five vaccinated dogs, lot D, also given the same dilution of test virus, died on the 15th and 19th days (40 per cent). Two of the control lot, H, succumbed to the 1:40,000 dilution of virus (50 per cent).

In this test (Table VI), the mortality of the controls was 65 per cent as compared with 80 per cent and 40 per cent of the two vaccinated lots respectively, showing clearly, as in Experiments 2 and 3 above, that dogs vaccinated with commercial rabies vaccines do not withstand the least amount of test virus fatal to less than 100 per cent but more than 50 per cent of controls.

The results of all experiments to date on the immunizing potency of

commercial canine vaccines given to dogs according to directions are combined in Table VII. Of the groups receiving two to four times the least dose fatal to nearly 100 per cent of controls, the thirty-seven non-vaccinated animals showed a mortality of 100 per cent, the thirty-nine given phenolized vaccines, 82 per cent, and the thirty-six given chloroformized

TABLE VI

Immunizing Effects of Canine Antirabies Vaccines on Beagle Dogs

Experiment 4, Test 10

Treatment of dogs	Dilution of test virus	Fate of dogs inoculated into the neck muscles (right and left) with 0.25 cc. of dog passage virus, 15811		
		Day of death following inoculation	No. dead/ No. injected	Dead
				per cent
A. No vaccine	1:400	12, 19, S	2/3	66
B. " "	1:4,000	15, 16, 18, 30, S	4/5	80
C. No. 10 phenolized vaccine	1:4,000	14, 16, 17, 43, S	4/5	80
D. No. 1 chloroformized vaccine	1:4,000	15, 19, S, S, S	2/5	40
H. No vaccine	1:40,000	39, 42, S, S	2/4	50

S = animal remained well following injection. Survivors discarded after 50 days.

TABLE VII

Summary of Mortalities of Dogs Vaccinated with Commercial Canine Vaccines and Tested Subsequently with an Intramuscular Injection of Dog Passage Virus, 15811

Vaccine employed	Mortality from test virus in dilutions			
	1:50 to 1:200 (2 to 4 × least dose fatal to 93 per cent of controls)		1:400 to 1:4,000 (2 to 20 × least dose fatal to 51 to 99 per cent of controls)	
	No.	*per cent*	*No.*	*per cent*
None	37 of 37	100	30 of 38	79
Phenol	32 of 39	82	9 of 13	70
Chloroform	19 of 36	53	4 of 10	40

vaccines, 53 per cent. Again, of the groups receiving a still smaller amount of test virus (two to twenty times the least dose fatal to less than 100 per cent but more than 50 per cent of controls), the thirty-eight non-vaccinated animals showed a mortality of 79 per cent, the thirteen given phenolized vaccine, 70 per cent, and the ten given chloroformized vaccines, 40 per cent. These differences between vaccinated and non-vaccinated dogs are negligible.

Immunizing Potency of Commercial Canine Antirabies Vaccines Administered in Larger Doses and by the Intraperitoneal Route

Concurrently with the above experiments, tests have been made of the immunizing potency of canine vaccines administered in larger doses and by the intraperitoneal rather than the subcutaneous route. Previous tests in mice (1) had shown that chloroformized vaccines are capable of conferring a degree of immunity, provided two to five times the comparable dose per gram of body weight is given and the injections are made intra-

TABLE VIII

Immunizing Effects of Canine Antirabies Vaccines on Beagle Dogs

Experiment 5, Test 4

Treatment of dogs	Dilution of test virus	Fate of dogs inoculated into the neck muscles (right and left) with 0.25 cc. of dog passage virus, 15811		
		Day of death following inoculation	No. dead/ No. injected	Dead
				per cent
A. No vaccine	1:50	13, 15, 15, 17	4/4	100
B. Vaccine 3: Chloroform, 1 dose, 20 cc., iper.	"	34, 36, S, S, S	2/5	40
C. " 3: Chloroform, 1 dose, 10 cc., iper.	"	S, S, S, S	0/4	0
D. " 3: Chloroform, 2 doses, 5 cc. each, iper.	"	22, S, S, S	1/4	25
E. Vaccine 3: Chloroform, 1 dose, 10 cc., subc.	"	22, 23, 54, S, S	3/5	60
F. " 3: Chloroform, 2 doses, 5 cc. each, subc.	"	19, S, S	1/3	33
G. No vaccine	1:500	12, 14, 15, 27, 52	5/5	100
H. " "	1:10,000	15, 18, 18, 23, 31	5/5	100

S = animal remained well following injection. Survivors discarded after 70 days.

peritoneally instead of subcutaneously. The following protocol illustrates the type of experiment and results obtained in dogs.

Experiment 5.—Four dogs, lot A, remained unvaccinated. Five dogs, lot B, received chloroformized vaccine No. 3 in one dose of 20 cc. intraperitoneally. Four dogs, lot C, received the same vaccine in one dose, 10 cc., intraperitoneally; four dogs in lot D received the vaccine in two doses of 5 cc. each intraperitoneally; five dogs in lot E each received one dose, 10 cc., subcutaneously, and three dogs, lot F, two doses of 5 cc. each subcutaneously. Five dogs in lot G and five in lot H remained unvaccinated. 3 weeks later each dog in lots A to F inclusive received 0.25 cc. of virus 15811, diluted 1:50, into the neck muscles of each side. Dogs in lot G received the same dose of a 1:500 dilution, and lot H, a 1:10,000 dilution.

The results of the test are shown in Table VIII. The lot A controls died of rabies on

the 13th, 15th, 15th, and 17th days respectively (100 per cent). Two of the five dogs in lot B, given 20 cc. of vaccine intraperitoneally, died on the 34th and 36th days (40 per cent). No dogs in lot C, given 10 cc. of vaccine intraperitoneally, succumbed (0 per cent). Only one of four dogs in lot D given two 5 cc. doses intraperitoneally died (25 per cent). Three of five dogs in lot E, given 10 cc. of vaccine subcutaneously, died on the 22nd, 23rd, and 54th days respectively (60 per cent). One of three dogs given two doses of 5 cc. each subcutaneously died on the 19th day (33 per cent). Five controls given the 1:500 dilution of test virus died on the 12th, 14th, 15th, 27th, and 52nd days (100 per cent). Five dogs given the 1:10,000 dilution of test virus likewise died on the 15th, 18th, 18th, 23rd, and 31st days (100 per cent).

The test shows (Table VIII) that chloroformized vaccine immunizes dogs fairly well (ten of thirteen, 77 per cent), if given intraperitoneally in at least double the standard 5 cc. dose. If given subcutaneously in similarly large doses, the result, although less striking (four of eight, 50 per cent), is still significant. This protection was obtained against a large amount of test virus—in this single test, at least 200 times, although in average tests not more than four times, the least dose fatal to 100 per cent.

DISCUSSION

The development of a quantitative method for measuring the immunizing potency of antirabies vaccines in dogs affords an opportunity to test rabies vaccines critically under conditions approaching those in nature. The failure of commercial phenolized and chloroformized canine vaccines to immunize dogs under the above experimental conditions weighs against their present value in the field. On the other hand, the fact that chloroformized vaccines in larger doses, administered intraperitoneally, successfully immunize suggests that the development of a potent, practical vaccine is not an impossibility.

The dog test has likewise been useful in checking the results of the mouse test (1). Phenolized vaccines found to be negative in mice proved likewise negative in dogs and chloroformized vaccines, equivocal or irregular in mice, proved the same in dogs. Finally, larger doses of chloroformized vaccines injected intraperitoneally proved effective, though irritative, in both mice and dogs. Thus the results of the dog test have paralleled those of the mouse test.

The parallelism between results in dogs and those previously reported in mice (1) establishes the reliability of the mouse test as an index of the immunizing effect of rabies vaccines in other animal species. Accordingly the mouse test becomes a simple, inexpensive, and practical tool for the testing of routine or experimental vaccines.

CONCLUSIONS

1. A quantitative method is described for testing the immunizing potency of antirabies vaccines in dogs.

2. Phenolized, single-injection, canine vaccines from seven manufacturers, when administered to dogs according to directions, failed to protect them against the least measurable amount of test virus fatal to 50 per cent or more of controls. Chloroformized vaccines from two of three manufacturers, under the same conditions, gave equivocal or suggestive results.

3. Commercial chloroformized vaccines in 10 cc. doses, injected intraperitoneally rather than subcutaneously into dogs, conferred a significant degree of immunity but proved temporarily irritative to the peritoneum.

4. These results of canine vaccines in dogs parallel closely those already reported in mice.

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EXPLANATION OF PLATE 35

FIG. 1. Negri bodies in Ammon's horn of W-Swiss mouse injected intracerebrally with the brain of a non-vaccinated dog in Experiment 10, which had been inoculated intramuscularly with rabies strain 15811.

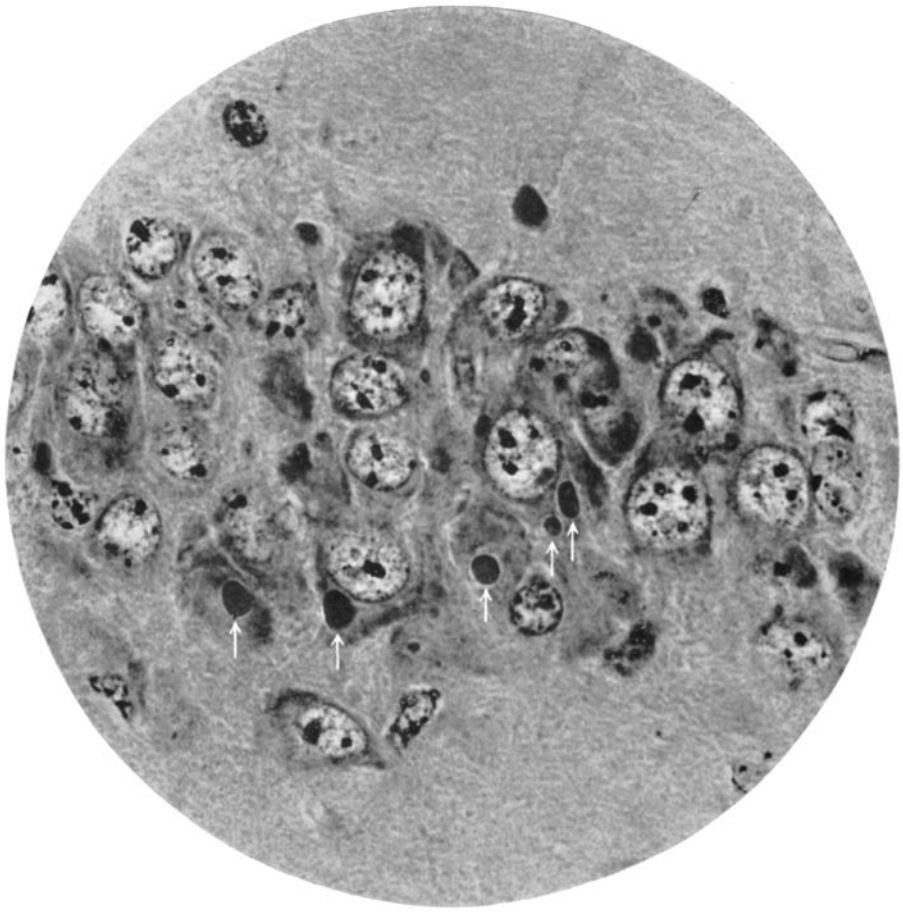

FIG. 1

Photographed by Joseph B. Haulenbeek