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Persistence of Neutralizing Antibody for a Year following Vaccination of Monkeys with Lansing Poliomyelitis Virus.* (18180)

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Whenever the question of vaccination against an infectious disease arises, two primary questions are asked. First, is there a safe, effective vaccine available; and second, is the immunity induced sufficiently lasting to achieve the desired result? For poliomyelitis, these questions are so far still under discussion as regards the experimental disease. A procedure for immunizing monkeys with active poliomyelitis viruses has been described (1), which however is not without risk to the experimental animal(2). After repeated intramuscular injections of sufficient doses of active virus, rhesus monkeys became immune to the extent that they showed high concentration of circulating antibody and resisted a large intracerebral dose of the same active virus. Furthermore, the level of circulating antibody associated with immunity to intracerebral resistance has been defined(3). The intracerebral resistance and the neutralizing antibody were found by cross testing with various types of poliomyelitis virus to be specific for the immunological type of poliomyelitis virus used for immunization(2,4,5). Having available an effective means for immunizing monkeys, the next important step was to find how long such immunity would last. Three rhesus monkeys which had been immunized with active Lansing poliomyelitis virus in the course of earlier work(3) were available for study of circulating antibody. After a course of intramuscular vaccination, all three individuals proved immune to intracerebral challenge with a large dose of Lansing virus. Following an additional intramuscular dose of virus, the monkeys were bled at two months' intervals for a year. At the end of the year, they received a single booster dose of virus intramuscularly, and

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2. Morgan, I. M., *Am. J. Hyg.*, 1949, v49, 225.

3. Morgan, I. M., *J. Immunol.*, 1949, v62, 301.

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TABLE I. Endpoints of Anti-Lansing Neutralizing Antibody in Serum of Monkeys During the Year Following the Last Intramuscular Vaccination Dose.

Mo. after vaccination						0	2	4	6	8	10	12	1	4	5
	1947						1948							1949	
	9/16	9/16,23	10/7,23	10/30	11/21	11/28	1/19	3/23	5/19	7/16	9/16	11/19	12/17	3/9	4/18
		↓↓	↓↓	i.c.	↓				1.4*			↓			
C993	0			3.6	—	3.1	2.2	2.0	1.5	1.0	1.4	1.5	3.4	2.4	2.5
C996	0			2.5	1.9	3.5	3.0	2.2	2.0	2.0	1.6	1.5	3.5	2.5	2.7
						2.8			2.7						
C998	0			2.4	2.2	3.3	3.5	2.9	2.7	1.8	2.5	2.3	3.0	1.7	2.4

↓ Monkeys received by intramusc. inj. 1 cc Lansing monkey spinal cord pools, as follows: On Sept. 16, 23, and Oct. 7, 23, 1947, rhesus C993 received a 5% suspension of pool IX(7); C996 and C998, a 2.5% suspension. On Nov. 21, all 3 monkeys received a 20% suspension of pool VII (C193). On Nov. 19, 1948, all 3 received 10% suspension of pool VII (C193).

i.c. On Oct. 30, 1947, all 3 monkeys were given intrathalamic challenge dose of 0.8 cc 10% suspension of pool IX(7). None showed signs of poliomyelitis.

* Duplicate figures indicate result of repetition of the intracerebral mouse neutralization test. Results reported as negative log of serum dilution at LD_{50} (before mixing with an equal volume of mouse brain virus suspension containing 20 LD_{50}).

were bled three more times in the following 5 months. The results of intracerebral mouse neutralization tests on these various sera will be reported and discussed here.

Materials and procedures. Three *M. mulatta* in good physical condition, weighing between 5 and 7 lb proved tuberculin negative to an intradermal injection of O.T. in the upper eyelid. The vaccination experience of these 3 individuals (C993, C996 and C998) in a series receiving graded vaccination doses has already been reported(3). The schedule of injections into the calf muscles (alternating sides) is given under Table I. The 50% paralyzing dose of Lansing pool IX(7) was 4.8 by intracerebral titration in monkeys; the PD_{50} of pool VII (C193) = 5.3 as reported earlier(3). Five months following the last vaccination dose (the end of the period shown in Fig. 1 and Table I), all received an intrathalamic challenge with 0.8 cc of 10% suspension of Lansing pool IX (D186). This pool has not been titrated, but was made under conditions which have regularly yielded pools with PD_{50} = 4.8 to 5.3. Two normal rhesus controls were injected simultaneously with the same suspension. Samples of heart's blood were taken from all 3 monkeys before vaccination, 6 weeks after beginning vaccination (just before intracerebral challenge) and again 3 and 4 weeks after intracerebral challenge. From the time of the last intramuscular vaccination dose (given 3 weeks after intracerebral challenge) blood samples were taken at two months' intervals for a year. After the intramuscular step-up dose at the end of that period, 1, 4 and 5 months' samples were obtained. During their residence of over a year and a half in the animal house, C993 and C996 remained in excellent physical condition. C998 developed diarrhea twice, but recovered on treatment with carbasone and sulfadiazine. Serum endpoints were measured by intracerebral mouse neutralization tests, according to the method used previously(7). Mixtures of 10-fold dilutions of serum in saline were made with equal volumes of a dilution of Lansing mouse CNS suspension (brain stem and spinal cord). The dilution of virus was such that the injecting dose of 0.03 cc contained 10 LD_{50}. Serum endpoints of 50% mortality of mice were calculated according to Reed and Muench(8).

Experimental results. Table I summarizes the experience of the three monkeys with Lansing virus and shows the endpoints found

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8. Reed, L. J. and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

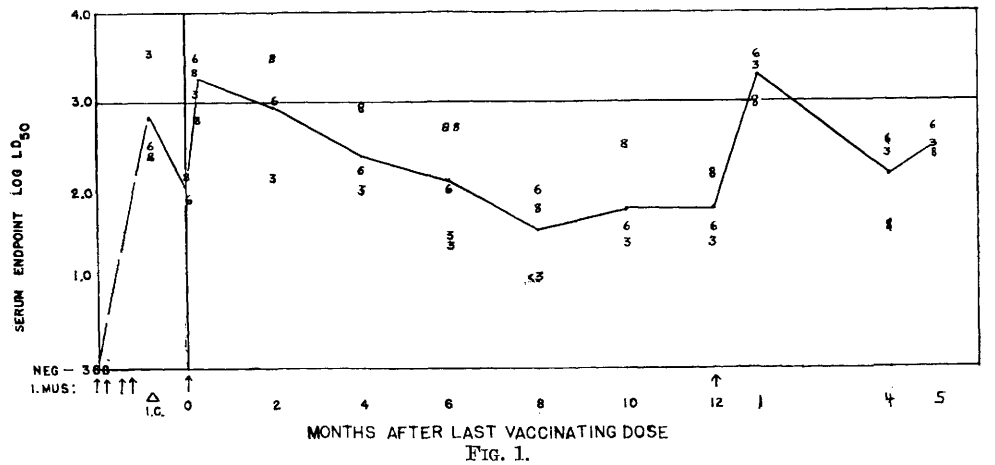

FIG. 1.

Duration of serum antibody in monkeys for the year following last intramuscular vaccination dose.

by serum neutralization tests. A few tests run in duplicate indicate differences in endpoints with a given serum of 0 to 0.5 log which is consistent with previous experience on reproducibility(7). The serum endpoints are represented graphically in Fig. 1. A line has been drawn through the mean values of the endpoints of the three sera taken at a given time. It can be seen that the individual endpoints of the 3 sera did not vary markedly from each other at any given time. A horizontal line has been drawn through the level of serum endpoint = 3.0, since it had been found in earlier experience(3) that serum endpoints in the range of 3.0 to 3.6 were optimum after such a course of vaccination. Such levels of serum antibody were associated with 100% immunity (in 37 monkeys) to the intracerebral challenge with virus. In the range of serum endpoints from 2.0 to 3.0 about half the individuals resisted. Although the 3 monkeys considered here received somewhat less than the optimum concentration of virus suspension required to insure the highest level of immunity, all three had survived intracerebral challenge and thus became available as resistant animals for study of duration of immunity.

An additional intramuscular vaccination dose of active virus 3 weeks after the intracerebral challenge boosted the serum endpoints in all three animals into the optimum range of 3.0 and over. Thereafter, as shown by serum neutralization tests done at 2 months' intervals, the serum levels fell gradually until 8 months. For the last 4 months of the year the endpoints remained at a level with a mean at about 1.8. Optimum serum endpoints were again attained in all 3 in response to an intramuscular booster dose at the end of the year. At 4 and 5 months after this booster dose, the endpoints again dropped to a moderate level.

To complete the history of these monkeys, beyond that shown in Fig. 1 and Table I, it should be added that at 5 months after the final booster dose, all 3 received an intracerebral challenge with active Lansing virus. Two normal rhesus monkeys, E854 and E855, inoculated at the same time showed paralysis on the 7th and 5th days after intracerebral injection. C993 and C996 showed no signs of poliomyelitis, but C998 developed paralysis beginning 7 days after challenge. This paralytic rate of one out of 3 vaccinated individuals with serum antibody levels ranging from 2.4 to 2.7 is consistent with earlier experience with a larger number of monkeys (3). With antibody levels in this intermediate range, about half of the group of monkeys became paralyzed following challenge. C993 and C998 were sacrificed one month after intracerebral challenge, whereas C996 died from unknown cause. From the 2 individuals sacrificed, samples of serum and spinal fluid, as well as suspensions of anterior horn of the

spinal cord, and visual cortex from the occipital pole of the brain were studied for antibody levels. C993 showed levels consistent with those already reported in monkeys vaccinated and challenged with Lansing virus without developing paralysis(9,10). C998 showed levels characteristic of a vaccinated individual who nevertheless succumbed to typical poliomyelitis on challenge. In C993 the endpoints of serum, spinal fluid, anterior horn and visual cortex were 2.6, 0.5, 0 and 0.7 respectively; in C998 they were 3.2 (no spinal fluid sample), 4.2 and 1.8. The greatest difference was obviously in the anterior horn of the spinal cord. In the former individual which showed no poliomyelitis, there was no antibody found in this susceptible area, whereas in the latter, convalescing from poliomyelitis, highest levels were found in this area which had been infected, as consistent with earlier findings(9,10). The serum levels found at the time of sacrifice have not been included in the table since an intracerebral rather than intramuscular injection of virus intervened. In C993 the serum endpoint did not change after intracerebral challenge (2.5 before and 2.6 after challenge). This is similar to our previous findings in a total of 6 monkeys which had also been vaccinated with Lansing virus. Serum antibody levels were found to be the same before and after intracerebral challenge with Lansing virus. In this regard, the serum of C998, which rose from 2.4 to 3.2 is an exception for which there is no apparent explanation.

Discussion. Having achieved a method of effective immunization of monkeys with poliomyelitis viruses, it became possible to study the vital question of duration of such immunity. Monkeys vaccinated intramuscularly to the extent that their sera showed optimum levels retained demonstrable serum neutralizing antibody during a year free from vaccination. The levels fell gradually during the first two-thirds of the year; in the last third, a moderate level was maintained. A single booster dose at the end of the year raised the serum levels of all three into the optimum range once more, from which they again fell in the first third of the second year.

These findings, although based on a small number of animals, are encouraging towards the goal of inducing effective, enduring immunity to poliomyelitis. However it must be remembered that the procedure used in inducing this immunity was not without risk to the experimental animals(2) and therefore cannot be considered for human use. It should be added that monkeys have also been vaccinated with poliomyelitis viruses inactivated with formalin to the extent that no remaining active virus was demonstrable(11). In the monkeys vaccinated with inactive virus, antibody levels fell more rapidly than in the present series of monkeys vaccinated with active virus.

Summary. The level of serum neutralizing antibody to Lansing poliomyelitis virus has been followed in three rhesus monkeys. After a course of intramuscular vaccination with active virus, which rendered the animals resistant to intracerebral challenge with a large dose of virus, they received a final vaccinating dose intramuscularly. During the year following this final dose, no more vaccine was given. Their antibody levels, optimum at the start, fell gradually during the first 8 months, then remained at a moderate level for the last 4 months. At the end of the year, a booster dose of active virus given intramuscularly raised their antibody to optimum levels once more, from which they gradually drifted downward.

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