Passive Immunity in Poliomyelitis VI. Antibody Decline in Rhesus Monkeys Inoculated with Homologous Lansing Antiserum.* (20154)

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We have previously reported that the intramuscular inoculation of monkey immune serum conferred a significant degree of protection on rhesus monkeys challenged intracerebrally with 50 or 100 PD_{50} of Lansing virus(1). These animals were bled at weekly intervals for 6 weeks following the inoculation of serum in order to correlate the outcome of challenge with serum antibody titer, for it appeared probable from the studies of Morgan(2) that a titer of 3.0 would ensure protection. As an additional contribution to the study of passively administered poliomyelitis antibody in monkeys, we now report our findings in a group of 9 rhesus monkeys that survived a cerebral challenge with Lansing virus. The antibody titers have been determined on surviving monkeys in a passive protection experiment previously referred to as Exp. No. 2(1). Bodian(3) has reported that the intramuscular inoculation of human gamma globulin containing poliomyelitis antibodies protected a significant percentage of rhesus monkeys against intramuscular challenge with poliomyelitis strains. In a group of uninfected animals inoculated with gamma globulin, Lansing antibody declined at a constant logarithmic rate.

Materials and methods. Details of passive protection experiment. The details of Exp. No. 2 have already been given(1). To summarize, 20 control rhesus monkeys were inoculated intramuscularly with 5 ml of normal rhesus serum per lb of body weight. A group of 20 monkeys of similar weight received the same dosage of Lansing hyperimmune rhesus serum (titer 3.5 in mice). On the day following the inoculation of serum, all 40 animals were challenged by the thalamic route with 50 PD_{50} of Lansing virus monkey cord pool. Small amounts of blood (5 ml) were taken from all monkeys the day prior to the inoculation of serum and at weekly intervals thereafter, for 6 weeks. Of the 20 monkeys in the control group, one died of bacterial meningitis; 18 of the remaining 19 developed typical prostrating paralysis. The single monkey that escaped paralysis (A409) showed no histologic evidence of poliomyelitis. Of the 20 monkeys inoculated with immune serum, 3 died of bacterial meningitis and one of diarrhea. Only 4 of the remaining 16 developed poliomyelitis; the 12 surviving animals showed no clinical or histologic evidence of poliomyelitis. *Antibody determinations.* Monkey sera were tested for the presence of Lansing virus neutralizing antibody by cerebral inoculation (0.03 ml), in groups of 8-10 mice (12-14 g), of mixtures of 10-fold dilutions of unheated serum and enough Lansing mouse pool virus to give a final 100 LD_{50}. This technic has been employed in these laboratories for several years. The mice were observed for 4 weeks, and the 50% neutralizing endpoints calculated by the Kärber method(4).

Results. Antibody titers in monkeys receiving normal monkey serum. The serum of monkey A409, that failed to develop paralysis when challenged, neutralized Lansing virus (serum titer 1.6); a similar result was obtained when heat-inactivated serum was tested. The sera of all other monkeys were free of Lansing antibody. *Antibody titers in monkeys receiving immune monkey serum.* Four animals in this group died from nonspecific causes and their sera were not tested further. The antibody titers in the 4 monkeys that developed prostrating paralysis, and in 9 of the 12 that remained healthy, are shown in Table I (antibody levels were not determined on the remaining 3 monkeys). All sera obtained before inoculation were free of Lansing antibody. The average titer of sera obtained one week after inoculation in

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TABLE I. Lansing Antibody Titers in Monkeys in Passive Protection Experiment (No. 2).

Reference No. of monkeys	Result of cerebral challenge (50 PD_{50} of Lansing virus)*	50% serum titers at following periods†					
		Before inj. of serum	Wk after inj. of serum				
			1	2	3	4	6
A423	Prostrating paralysis	0	1.0				
424		0	2.1	1.75			
437		0	2.0	1.6	1.2		
442		0	2.4	1.8			
Avg 50% serum titers:			1.87	1.72			
A425	No evidence of poliomyelitis clinically or histologically	0	2.1	1.9	1.3	1.3	.4
426		0	1.4	1.0	1.0	.9	−.2
427		0	2.2	2.0	1.0	.8	.5
428		0	1.9	1.3	.8	.4	−.1
430		0	1.8	1.0	1.5	.5	.5
431		0	2.2	1.1	.9	1.0	.0
432		0	1.5	1.7	1.2	1.2	.8
433		0	2.2	1.9	1.6	.8	.7
434		0	1.6	1.0	1.1	.6	.0
Avg 50% serum titers:			1.88	1.43	1.16	.83	.29

* Challenge carried out on day following inoculation of 5 ml normal or immune serum/lb body wt.

† Titers expressed as negative logarithms.

the 4 monkeys that became paralyzed was 1.87, and in the 9 monkeys that did not develop paralysis, 1.88. In these 9 monkeys, titers fell to a level that was just detectable 6 weeks after injection of antiserum. The average titers in the 9 monkeys mentioned are plotted in Fig. 1, from which it may be seen that the relationship between the average titers and the time following the injection of antiserum is linear over the period one to 6 weeks, the concentration of antibody falling at a constant logarithmic rate of 10.2% per day. If one assumes that the antibody declined at the same rate during the first week following injection of serum, then the half-life would be 6.8 days, and, by extrapolation, the initial serum titer would be approximately 2.1.

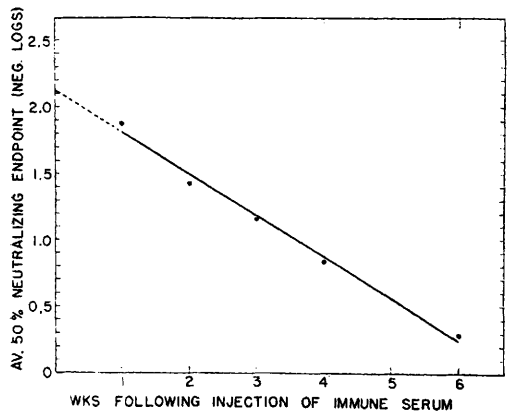

FIG. 1. Rate of decline of Lansing antibody in serum of 9 rhesus monkeys challenged by the cerebral route with Lansing virus in passive protection experiment, and failing to develop evidence of poliomyelitis.

Discussion. No essential difference was found between the average neutralizing titers of sera obtained one week following the injection of antiserum in 9 monkeys that remained healthy following challenge, and in 4 that developed poliomyelitis. An initial antibody titer of approximately 2.1 was sufficient to protect 9 of 13 challenged monkeys. Presumably, therefore, a titer in excess of this figure would protect all monkeys challenged with 50 PD of Lansing virus. The rate of antibody decline in the 9 rhesus monkeys inoculated with Lansing immune rhesus monkey serum, and which survived a cerebral challenge without developing clinical or histologic evidence of poliomyelitis, was almost exactly the same as in a number of normal monkeys receiving human gamma globulin in the experiments of Bodian(3). The half-life of the passively administered antibody was 8.0 days in the study of Bodian and 6.8 days in our experiment. A similar figure (6.6 days) was obtained by Dixon *et al.*(5), who studied the

elimination in rhesus monkeys of homologous gamma globulin labelled with I^{131}.

In the study of Bodian, the initial serum antibody level was found to be approximately 2.0 in monkeys receiving 10 ml of human gamma globulin per kilo body weight. A similar initial serum antibody titer was determined by extrapolation in our experiment in which the monkeys received a corresponding dosage. The Lansing antibody titer of the gamma globulin used by Bodian was 2.8, and the immune monkey serum used in our study had a titer of 3.5. It should, however, be realized that the 2 studies are not precisely comparable, because the monkeys used by Bodian were normal, whereas our monkeys had received an injection of Lansing virus intracerebrally.

Our observations on the fate of Lansing antibody in rhesus monkeys were made as part of a wider program on passive immunity, in which experiments were carried out on humans. In a study previously reported(6), we found that when human gamma globulin containing Lansing antibody was administered to 6 children and 4 adults, whose serum was initially free, antibody could be detected in small amounts up to the 6th week following injection. It is of considerable interest that in the extensive field trials of Hammon *et al.* (7-9), human gamma globulin showed a protective effect against the development of paralytic poliomyelitis for 5 to 6 weeks. These observations suggest that the half-life of poliomyelitis antibody passively injected into humans is considerably longer than the period of 7 to 8 days for the same antibody injected in rhesus monkeys. In the studies of Dixon *et al.*(5), the half-life of human gamma globulin was found to be approximately 13 days for adults, and 20 days for children up to 8 years of age. The half-life of placenta-passing Rh antibodies in newborn infants was found by Weiner(10) to be approximately 30 days.

Summary. 1. Serum antibody titers have been determined in a passive protection experiment in which rhesus monkeys were inoculated intramuscularly with Lansing antiserum prepared in rhesus monkeys; the monkeys were challenged thalamically with 50 PD_{50} of Lansing virus. 2. Four of these monkeys developed paralysis, and 9 remained clinically and histologically normal. 3. Average serum antibody titers in samples removed one week after inoculation were $10^{-1.87}$ in the paralyzed group, and $10^{-1.88}$ in the unaffected group. 4. By extrapolation, the initial antibody titer was estimated to be $10^{-2.1}$. The average titer 6 weeks after inoculation was $10^{-0.29}$. 5. The rate of decline of antibody in the 9 monkeys that survived the cerebral challenge was constant, the half-life being 6.8 days.

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