THE GROWTH CURVE OF THE LANSING STRAIN OF POLIOMYELITIS VIRUS IN THE CENTRAL NERVOUS SYSTEM OF THE MOUSE*

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Bodian and Cumberland have described the growth curve of the Lansing strain of poliomyelitis virus in the *rhesus* monkey (1). Although the mouse is used in most laboratories for the study of poliomyelitis virus infection, the pattern of multiplication of virus has not been studied in this species. The present report deals with a description of this aspect of the pathogenesis of experimental poliomyelitis in mice.

Materials and Methods

Virus.—A pool of the 85th mouse passage, Lansing strain (2) of poliomyelitis virus which has been maintained in this laboratory[1] was used for all but the first experiments in which the 80th and 82nd mouse passages were used. The pools consisted of the entire central nervous system (CNS) of mice paralyzed within 6 days of intracerebral inoculation with virus, and of spinal cords and medullae (to be referred to subsequently as cords) of mice paralyzed thereafter. The infectivity titers of the pools measured by repeated intracerebral tests were between $10^{-4.0}$ and $10^{-4.5}$ LD_{50} (50 per cent lethal dose). No significant differences were noted between titrations performed with 20 and with 8 mice per dilution.

Suspensions containing virus were prepared in all instances by grinding the tissues with a small amount of alundum in a mortar with a pestle, with the addition of sterile saline (0.15 M NaCl). They were centrifuged at 2000 R.P.M. for 30 minutes, and the sediments discarded. The supernatant fluids, which thus constitute the preparations of virus, were usually tested for bacterial sterility before use. In some tests it was desired to inoculate mice without delay, so crystalline potassium penicillin G (final concentration 50 u/ml.) and streptomycin sulfate (final concentration 0.1 mg./ml.) were added to the suspensions in saline. No disturbing bacterial growth resulted in mice receiving the materials and the antibiotics had no effect on the resulting titer of virus.

Growth Curves.—White Swiss mice, 3 to 5 weeks of age, were inoculated intracerebrally with 0.03 ml. of either a 10 per cent or 1 per cent suspension of virus. They were then closely observed for signs of infection. Groups of 5 to 10 unparalyzed or paralyzed mice were sacrificed with ether at various intervals from immediately after, to 10 days after inoculation and

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[1] In Dr. Armstrong's laboratory this strain had been through 14 transfers in monkeys, 1 in cotton rats, and several in mice. It was subsequently maintained by mouse passage in Dr. S. D. Kramer's laboratory from which we received it, in the 16th mouse passage, in 1941. A description of some characteristics of this line has been made (8).

the brains and cords were harvested. The spinal cords and medullae were separated from the more rostral portions of the brain by severing just caudal to the pons. Brains and cords were stored in a chest, refrigerated with solid CO_2, and tissue suspensions were stored for 1 to 10 days at 4° C. Determination of the amount of virus in the material was then made by injection of a series of 10-fold dilutions in groups of 8 mice, each receiving 0.03 ml. of the material intracerebrally. Titers are expressed as the LD_{50} calculated according to the method of Reed and Muench (3).

RESULTS

In seven experiments titrations were made of the virus content of the brains and cords, separately, of mice sacrificed at various intervals after intracerebral inoculation of 10 per cent suspensions (approximately 2000 LD_{50}) of virus. The results are presented in Table I. Median values of all the experiments are shown in Fig. 1 and a single early growth curve after the inoculation of 10 per cent virus is illustrated in Fig. 2 (Experiment 4 in Table I).

Unparalyzed Mice.—Examination of the data reveals that after inoculation of a 10 per cent suspension, the amount of virus demonstrable in the brain diminishes during the next 6 hours to a level of less than 10 LD_{50}. Between the 6th and 9th hours a 20- to 50-fold increase in titer takes place so as to exceed that initially observed. Thereafter, the titer mounts progressively, although with smaller increments, to approach its peak of about 4.0 logs in approximately 24 hours. In 30 hours paralysis is detected in some of the animals.

In the cord the sequence is somewhat different. A consistently small amount of virus is detectable from the time of intracerebral injection until the 9th hour when an abrupt rise in titer takes place, parallelling that noted in the brain. In the next several hours the increase is small but at the 18th hour another marked rise in virus content is observed, and the level attained surpasses by 10-fold that of the brain of the same mice at that time. There is no great change between 18 and 24 hours but the titer at 30 hours may be higher (Experiment 7 in Table I). This coincides with the beginning of paralysis in the mice. The data suggest that the growth of virus in the cords of mice proceeds largely in two sharp bursts at intervals of 9 hours and the maximum amount of virus which appears in the cord is greater than that in the brain (Fig. 1).

After 30 hours, there was a tendency for the titers to be somewhat lower in the cords than those noted at 24 to 30 hours but they were usually maintained between 3.5 and 4.2 logs for 7 days. In the brains, however, the amount of virus detectable was not appreciably different irrespective of the time beyond the first 24 hours at which the mice were sacrificed.

Paralyzed Mice.—The titers of virus in the cords of mice which became paralyzed 2 to 10 days after inoculation were consistently higher than those in the cords of unparalyzed mice, with averages of 4.6 and 3.6 logs, respectively. This average titer was also higher than that of virus in brains of the same paralyzed mice, 3.9, or in brains of unparalyzed mice, 3.7. It is, of course,

impossible to know, under the conditions of the present experiments, which of the unparalyzed mice selected in the first 24 hours might have succumbed

TABLE I

Growth Curves of Virus in the Brain and Spinal Cord of Mice Inoculated with 10 Per Cent Suspension of the Lansing Strain of Poliomyelitis Virus

		Experiment No.	Titer of virus expressed as the negative log of the LD_{50} dilution																
			Hrs. after inoculation										Days after inoculation						
			0	3	6	9	12	16	18	24	30	36	2	3	4	5	6	7	10
Paralyzed mice	Brain	1											4.5	3.8	3.6			3.4	
		2											4.3	3.1	3.1	4.5	3.6		
		3											4.6	4.1	3.7	4.0	3.5	3.5	
		4										4.2							
		5									3.3	3.7	3.8	4.6			3.6		3.0
		6										4.4							
		7									4.1		3.8	4.6					
	Cord	1											4.8	4.6	5.0			4.7	
		2											4.0	4.2	4.4	5.2	5.0		
		3											4.6	4.5	4.8	4.5	4.6	4.7	
		4										5.5							
		5									4.8	4.7	4.5	5.0			4.7		3.9
		6										4.6							
		7									5.4		4.2	3.7					
Non-paralyzed mice	Brain	1								4.0			4.5	4.4	3.6	3.4		3.6	
		2												2.9	3.6	3.3	3.6		
		3				2.3			3.8	3.8			4.0	3.9	3.6	4.1	3.7	3.1	
		4	2.2	1.6	0.9	2.7	3.2		3.5	4.4		4.4							
		5			0.8	2.1	2.6		3.2	3.8	3.6	3.9		4.5			3.7		2.7
		6						3.3			4.2	4.3							
		7	1.4	1.0	0.6	2.3	2.4	3.1		3.9	4.1	3.5	4.0	3.6	3.7				
	Cord	1								5.3			3.3	3.8	3.8	4.2		3.4	
		2												2.8	4.0	3.4	4.0		
		3				1.2			4.4	4.5			4.1	3.0	3.1	4.5	3.9	3.7	
		4	0.6	0.7	0.9	2.1	3.0		4.3	4.3		4.1							
		5			0.5	1.8	2.3		4.4	2.6	4.5	3.6		3.4			4.6		2.9
		6						2.5			5.2	4.8							
		7	0.6	0.6	0.6		2.2	2.5	4.2	4.2	5.4	3.6	3.7	3.6	3.4				

shortly thereafter, and whether the initiation of virus multiplication in the brain and cord of mice which were to die late was considerably delayed in comparison with that in mice which would die early. The regularity of the growth curves obtained in the first 24 hours after inoculation, as seen in the different experiments, indicates, however, that in that period the multiplication

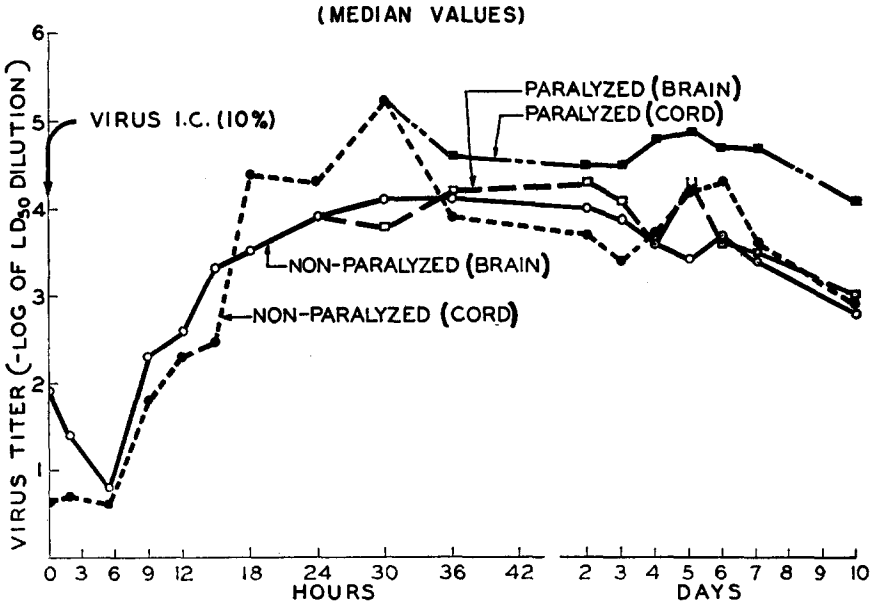

FIG. 1. Growth curves of virus in the brain and spinal cord of mice inoculated with the Lansing strain of poliomyelitis virus.

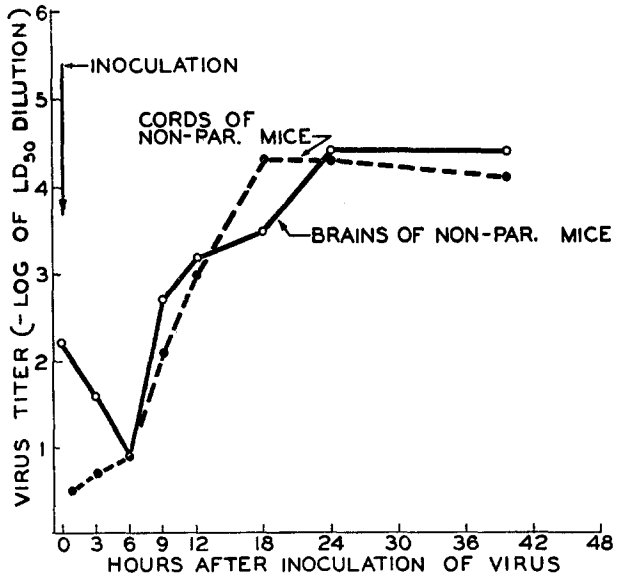

FIG. 2. Early phase of the growth curve of virus in the brains and cords of unparalyzed mice after intracerebral inoculation of a 10 per cent suspension of the Lansing strain of poliomyelitis.

of virus was rather uniform in mice irrespective of the time at which paralysis and death might ensue. It seems unlikely, therefore, that the mice which die later in the period of study, do so because of prolonged failure of the virus to begin multiplying. The amount of virus maintained in unparalyzed mice from 2 to 10 days after inoculation is by no means minimal but by most standards would represent a high titer of this virus; the mice are undergoing a well established infection without exhibiting paralysis. Nevertheless, the virus content of their cords is only 10 per cent of that found in the cords of mice becoming paralyzed at the same intervals after inoculation. It may be, then, that the increased amount of virus in the cord which distinguishes the para-

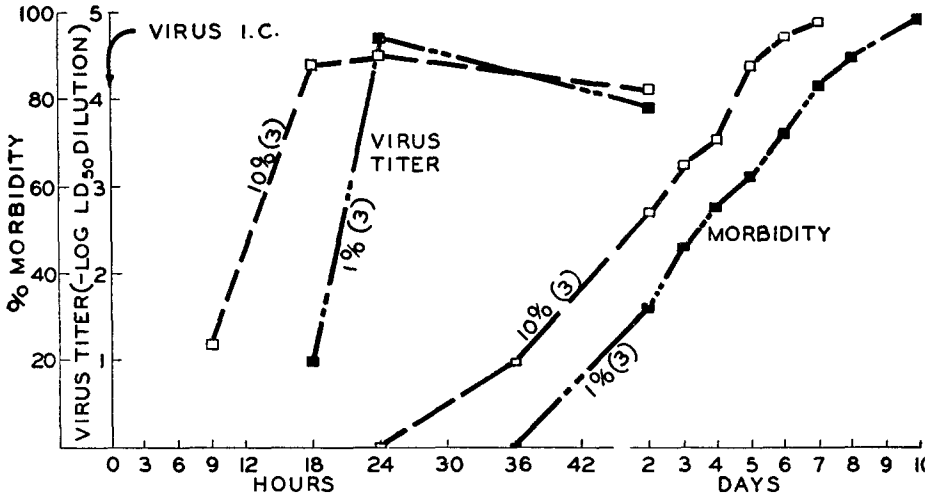

FIG. 3. Growth curves of virus in the spinal cords and of the morbidity of mice inoculated with two concentrations of the Lansing strain of poliomyelitis virus.

lyzed from the unparalyzed mice is a final accession which raises the titer to a paralytic, lethal level, and that the time of its development is determined by constitutional variation in the host population.

Effect of Dosage.—Fig. 3 presents the data obtained in a typical experiment for comparison of the growth curves in the cords of mice after intracerebral inoculation of 1 per cent and 10 per cent suspension of virus. Following the injection of 1 per cent virus, no decline in titer during the early hours is observed but the onset of the phase of rapid multiplication is delayed for 9 hours or more. Once the titers begin to rise, they increase at approximately the same rate to reach similar heights. The influence of the lag period persists, however, so that the interval between the times at which the highest titers are reached roughly corresponds to that between the onset of the initial growth phase.

It is also seen that the first mice with evident disease in the two groups

were detected 9 to 12 hours after the full titer of virus was reached in the cords of unparalyzed mice of the respective groups. After illness began, the rate at which mice of the two groups became paralyzed was essentially the same, but the time of onset of disease clearly reflects the differences in incubation periods with the two doses of virus.

DISCUSSION

Growth curves have been described for equine encephalomyelitis virus (4), influenza virus (5), and pneumonia virus of mice (6), in all of which an initial decrease in virus content was followed by a fairly steady rate of increase until a maximum point was reached after which there may be a plateau or a gradual decline in titer. Similar results have been reported with the Lansing strain of poliomyelitis virus in monkeys, in which maximum titers of virus were reached on the day before the onset of illness, followed by a rapid decline in titer after the onset of paralysis (1). The data in the present report show that the same curve of infectious titer was obtained in mice infected with the Lansing strain of poliomyelitis. It is difficult to know whether a decline also took place in mice during the period following paralysis, because of the brief interval between paralysis and death.

An earlier report from this laboratory of experiments with the Lansing strain of poliomyelitis virus showed that the titer of virus was approximately the same in the brains as in the cords of mice paralyzed within 5 days after intracerebral inoculation, but that after this time there was little or no virus recoverable from the brains although the virus content of the cord remained fairly constant (7). In contrast, the present experiments with the same line of virus indicate that virus persists in the brains of infected mice through the 10th day. This difference cannot be satisfactorily explained on the basis of present information. However, since it has recently been shown that the infectivity and incubation period of this line of the Lansing strain of virus have changed with progressive passage (8), it is not inconceivable that the persistence of virus in the brain after the 5th day, observed in the present study, represents another characteristic of the virus which has developed with passage.

The rapidity with which high infectivity titers are reached in the central nervous system of mice is of practical value in several ways. For example, a pool of virus can be prepared from the brains and cords of mice which are not yet paralyzed, thus avoiding the delays due to irregularities in the incubation periods as well as the loss of mice by death, which are disadvantages inherent in the usual method of collecting the central nervous system from only paralyzed mice. That this actually is of value was demonstrated by an experiment in which the brains and cords of more than 1000 mice, only 14 per cent of which were paralyzed, were harvested 48 hours after inoculation and the resulting pool was found to have a titer of $10^{-4.3}$.

In efforts to alter the course of poliomyelitis infection in mice, knowledge of the quantitative and temporal relations between multiplication of the Lansing strain of poliomyelitis virus and onset of clinical disease is both important and readily applicable. If mice are used for studies of prophylaxis of infection or prevention of disease by poliomyelitis virus the growth curve furnishes a proper basis for selecting the time in the course of infection at which to concentrate attention.

SUMMARY

After intracerebral inoculation of mice with a 10 per cent suspension (approximately 2000 LD_{50}) of the Lansing strain of poliomyelitis virus, the infectivity titer in the brain decreased for approximately 6 hours. It then rose rapidly for 12 to 18 hours to reach titers of over 10^{-4}. The rise in titer in the spinal cord closely paralleled that in the brain for 18 hours, after which the titer surpassed that in the brain by as much as one log. The infectivity titers in the central nervous system of unparalyzed mice remained between $10^{-3.5}$ and $10^{-4.2}$ for at least 7 days. With the onset of paralysis it was found that the titer was consistently and significantly higher in the spinal cords of paralyzed mice than in their brains or in the brains or cords of unparalyzed mice.

After inoculation of 1 per cent virus suspension the increase in titer occurred about 9 hours later than after the inoculation of 10 per cent virus suspension, and the onset of clinical signs of illness was also delayed. Once the titers began to rise, the rate was the same after the inoculation of either concentration of virus, and the maximal levels reached were the same. With both concentrations of virus, maximal infectivity titers in non-paralyzed mice were reached about 9 hours before the onset of signs of poliomyelitis.

The significance of these findings is discussed.

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