ADAPTATION OF A LANSING STRAIN OF POLIOMYELITIS VIRUS TO NEWBORN MICE*

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Young mice are more susceptible than older ones to the development of disease of the central nervous system (CNS) after introduction of several neurotropic viruses, especially into non-nervous tissues. A higher titer of some of the viruses is also found in the CNS of the young (1–9). In the earlier investigations animals shortly before or at the time of weaning fell into a group called young; with time the age limit in this young group was progressively lowered until in recent studies newborn mice from 1 to 3 or 4 days of age were found to be still more reactive to certain neurotropic viruses than those nearer the weaning period (5–8). An outstanding exception is the Lansing or rodent-adapted type of poliomyelitis virus. The susceptibility of mice to it becomes progressively enhanced with increasing age from the time of birth to 3 weeks (10–12).

It was assumed that if a Lansing-type poliomyelitis virus could be adapted to newborn mice and caused to multiply in their CNS to a degree equal to or greater than that in the adult CNS, progress might be made towards the solution of the problem of securing a practical specific complement-fixation antigen. In an earlier paper (13) it was shown that this assumption was justified, for a specific complement-fixation test had thereby been achieved.

The purpose of the present communication is to describe in detail how a Lansing-type virus was successfully adapted to the naturally resistant newborn mice so that they could eventually be infected regularly and uniformly, after a short incubation period, with results in infective titers of the central nervous tissues as high or higher than those obtained during the experimental disease in adult mice.

Materials and Methods

Virus.—The MEF1 strain of Lansing-type poliomyelitis virus was the one mainly used in this work, and finally chosen for the adaptation. This strain has been maintained by intra-

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cerebral mouse passage since its isolation in 1943 (14). The number of passages in mice is unknown, probably not more than 40. This Lansing-type strain of poliomyelitis virus is similar in every respect to the original Armstrong strain (15), except that in this laboratory it has given in general a more uniform response and slightly higher titers on inoculation into adult mice (16).

The Armstrong strain was also used in experiments designed first, to test the susceptibility of newborn mice to it, and secondly, to ascertain the identity of the adapted MEF1 strain in immunological and serological studies. Adaptation of the Armstrong strain to newborn mice was attempted but as soon as it became apparent that the MEF1 virus showed an increasing pathogenicity for newborn mice, the work with the Armstrong strain was pursued no further.

For convenience the virus propagated in young adult 3- or 4-week-old mice will be designated "standard" virus and that propagated in newborn mice "adapted" virus.

Mice.—W-Swiss mice (17) were used throughout. They were supplied by a single dealer as 3- to 4-week-old animals, and newborn mice were bred from them in this laboratory. The mice supplied by the dealer were held until 2- to 3-months old and then mated, 6 or 7 females and one male being placed in a cage. A few days before delivery was expected, the females were segregated into individual cages. They were observed twice daily and all births before 9 a.m. were counted as of a litter of the preceding day. About 8 young constituted an average litter. In this way a supply of 400 to 500 newborn a week was regularly secured.

Harvesting and Inoculation of Virus.[1]—Newborn mice injected intracerebrally received 0.02 ml. of inoculum through a 27 gauge, ¼ inch-long needle. The diluent was either 10 per cent normal rabbit serum in physiological saline solution or 0.2 per cent bovine plasma albumin in buffered saline (18). The CNS was collected by incising the skull and spinal column so as to expose the entire brain and cord. The average weight of the brain and cord of 6- to 9-day-old mice, at the time when they developed signs of the experimental disease following intracerebral inoculation of the tissues, was 0.26 gm; of this the brain alone weighed 0.2 gm. and the brain stem and cord, 0.06 gm.

EXPERIMENTAL

Relative Resistance of Newborn Mice to Intracerebral Inoculation of the Armstrong Strain.—Attempts to propagate the Armstrong strain of virus in newborn mice soon revealed that they were less susceptible than adult mice. This phenomenon was then studied systematically.

A 10^{-1} suspension of virus was prepared consisting of brain and cord from 3- to 4-week-old mice that were paralyzed following administration of the virus. This suspension was distributed in several screw-capped, nitrocellulose tubes, quickly frozen, and held at −25°C. As newborn mice of different ages became available, one of the tubes was opened and the virus suspension titrated by injection of groups of mice of different ages, including in each titration a group of young adult mice, 3- to 4-weeks-old as a standard for reference. 27 different titrations were carried out at 6 separate times with the results shown in Fig. 1.

It will be noted from Fig. 1 that while the infectivity titer of the virus tested in 21-day-old or older mice averaged $10^{-3.0}$, with limits of $10^{-2.5}$ and $10^{-3.5}$, it was lower, usually from 10^{-1} to 10^{-2}, in the newborn. In addition, the incubation period was more prolonged in the latter. In these respects the findings are in good agreement with those of Sabin (10) and Dalldorf (7).

[1] All such operations were done with the aid of deep ether anesthesia.

Adaptation of the MEF1 Strain to Newborn Mice.—In the first tests with the MEF1 virus it was apparent that newborn mice reacted to it in practically the

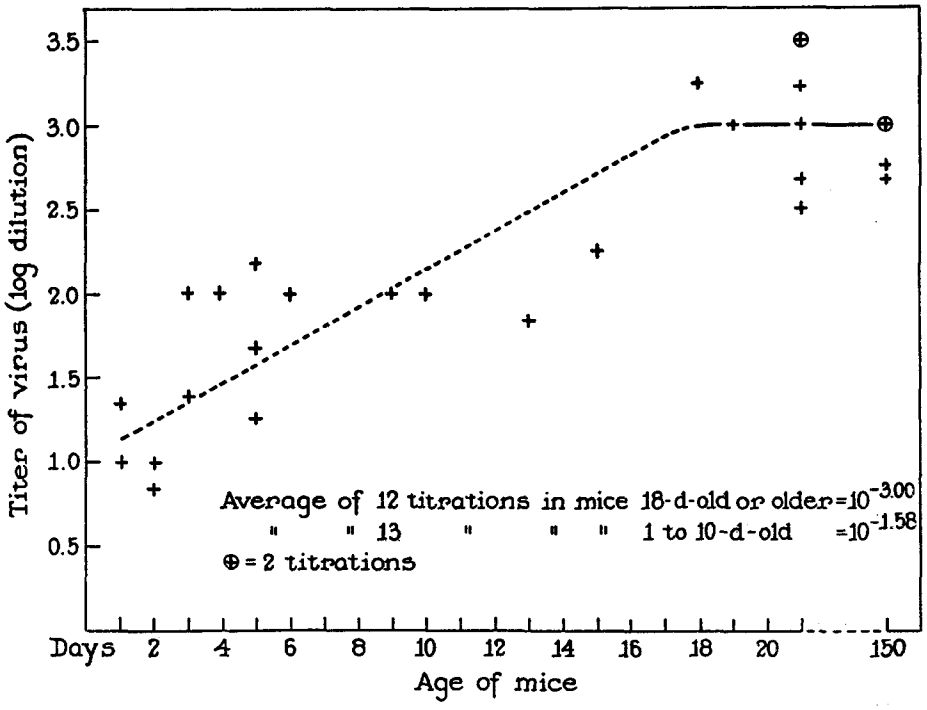

FIG. 1. Titrations of the Armstrong-Lansing poliomyelitis virus in mice of different ages.

TABLE I

Results of Intracerebral Inoculation into Newborn Mice of MEF1 Standard Virus, 10^{-1} Dilution

Experiment	Age	No.	Outcome of inoculation*
	days		
1	3	4	15,15,20,s
2	3	7	10,11,17,18,20,s,s
3	4	10	3,3,3,6,8,10,10,11,s,s
4	3	9	8,11,14,14,21,23,s,s,s
5	3	15	5,6,7,9,11,11,12,12,12,13,13,14,s,s,s

* 15 = paralyzed on 15th day after inoculation.
s = mouse showed no symptoms during a period of 25 days.

same way as to the Armstrong strain. That is, a number of them survived intracerebral inoculation of 10^{-1} dilution of virus and when they reacted, the incubation period was markedly prolonged, as shown in Table I.

The tabulated results, based on 5 different tests with the standard MEF1 virus of which 10^{-1} dilution was injected intracerebrally into 3- or 4-day-old mice, demonstrate that 34 of 45 of them, or 75 per cent, developed paralysis and other CNS signs and died within 25 days, the set period of observation. Moreover, the average duration of the incubation period of the affected animals was 11.7 days.

An attempt was then made to induce, if possible, a change in this virus, by the method of intracerebral serial transfer in newborn mice, so as to secure ultimately a greater degree of pathogenicity for this host. In the following account of the procedures used, the various steps taken are explicitly stated in minute detail for the reason that no precise formulation of a routine method or perhaps a more advantageous procedure can be now described. In other words, the manner of enhancing the pathogenicity of an infective agent by means of its consecutive passages conformed to general principles of bacteriology but the method here pursued could be regarded as purely empirical. The first 10 passages of the MEF1 virus in newborn mice are therefore given in still greater detail because no apparent evidence was derived from them to show that any change in the pathogenicity of the virus occurred during these early passages.

A 10^{-1} suspension was prepared of brain and cord from 3- to 4-week-old mice that were injected with the MEF1 standard strain; the suspension was given intracerebrally to 10 1-day-old mice (passage 1). On the 11th day after injection 2 were paralyzed, while the others remained without signs for 21 days. The CNS tissue of the 2 paralyzed mice was diluted 10^{-1} and this was similarly injected into 12 9-day-old mice (passage 2); all these developed CNS signs, one on the 5th day, the remaining ones from the 7th to 9th day. The CNS from a mouse paralyzed on the 5th day was injected intracerebrally into 16 3-day-old mice (passage 3); 10 of them were paralyzed or dead after incubation periods from 10 to 20 days. Passage 4 was derived from 2 mice, one of which was paralyzed and the other one normal looking on the 12th day after inoculation, and consisted of a new group of 15, of which 8 were 3 days old and 7 were 7 days old. They developed the experimental disease after incubation periods of 6 to 12 days which were similar in both groups; a total of 11 mice died within 21 days. The CNS of 3 mice, 1 paralyzed and 2 normal appearing, removed 6 days after inoculation, was injected into 24 mice aged 3 days (passage 5); 21 of them were paralyzed or dead within a 3 weeks observation period and had incubation periods from 5 to 13 days. A mouse paralyzed on the 6th day was employed for passage 6, containing 15 mice.

At this point, at passage 6, it was decided to continue the serial passages using CNS from mice on the 3rd or 4th day after injection whether sick or apparently well. As a rule, tissues from 4 animals were collected and only 3- or 4-day-old ones were used in subsequent passages. Of the 15 mice of passage 6, 4 which were normal looking on the 3rd day, were used for transfer. Of the remaining 11 held for observation during 3 weeks, only 6 showed paralysis and died after incubation periods of 5 to 19 days. In passage 7, 19 mice were inoculated; on the 3rd day, 4 normal appearing mice were employed for further passage and of the remaining 15, only 5 came down with signs, from the 4th to the 21st day. Passage 8 consisted of 17 mice and again none of the 4 mice selected for transfer were ill on the 3rd day; of the 13 remaining animals only 3 died from the 11th to 23rd day. In passage 9 were 17 mice; on the 3rd day after

inoculation one was paralyzed. This mouse and 3 others which were symptomless, were marked for subsequent transmission. Of the remainder, 11 showed signs from the 4th to the 16th day after injection. These data are summarized in Table II.

In the 10th and later passages the material for transfer consisted of the CNS of 4 mice of the immediately preceding passage, of which one, if not all, revealed the definite paralysis characterizing the experimental disease.

The results of the first 40 passages are illustrated in Figs. 2 and 3, with the exception of passage 2 for here the mice were 9 days old. The graphs exhibit groups of 10 passages in each, the total per cent of mice that developed signs

TABLE II

First Step in Adaption of the MEF1 Virus to Newborn Mice

Intracerebral inoculation, 0.02 cc. of 10^{-1} brain and cord

Passage No.	Age	No. inoculated	CNS used from mice		Outcome of inoculation*	Average incubation period of reactors
			Day of harvest	Paralyzed (P) or without signs (N)		
	days					*days*
1	1	10		Standard virus	11, 11, s, s, s, s, s, s, s, s	11.0
2	9	12	11	P, P	5, 7, 7, 7, 7, 7, 7, 7, 8, 8, 8, 9	7.8
3	3	16	5	P	10, 10, 10, 11, 11, 12, K12, 14, 17, 18, 20, s, s, s, s, s	13.3
4	3, 7	15	12	P, N	6, K6, K6, 8, 9, 11, 11, 11, 11, 12, 12, 12, 12, s, s	10.5
5	3	24	6	P, N, N	5, 5, 5, 5, 5, 5, 5, 6, 7, 7, 8, 9, 10, 10, 10, 12, 12, 12, 12, 13, 13, s, s, s	8.4
6	3, 4	15	6	P	K3, K3, K3, K3, 5, 8, 10, 10, 14, 19, s, s, s, s, s	11.0
7	4	19	3	N, N, N, N	K3, K3, K3, K3, 4, 8, 14, 14, 21, s, s, s, s, s, s, s, s, s	12.2
8	4	17	3	N, N, N, N	K3, K3, K3, K3, 11, 13, 19, s, s, s, s, s, s, s, s, s, s	14.3
9	3, 4	17	3	N, N, N, N	3, K3, K3, K3, 4, 5, 7, 7, 7, 8, 10, 10, 11, 15, 16, s, s	8.6

* 11 = mouse paralyzed on the 11th day after injection.
s = mouse remained well during 21 days.
K12 = normal appearing mouse killed on the 12th day after injection.

of the experimental disease after inoculation, and the cumulative per cent that reacted at any one day following injection of the virus. It is to be noted that during the first 10 passages in newborn mice no difference could be discerned in the outcome of the inoculation of virus propagated in the newborn and that of the standard virus. After the 10th passage in newborn mice, however, a change occurred as evidenced by the increase in the death rate, and particularly, by the shortening of the incubation period. For example, by the end of the 4th day after injection of the adapted virus 5 per cent of the mice were paralyzed or dead in the first 10 passages, 37 per cent in passages 11 to 20, 53 per cent in passages 21 to 30, 60 per cent in passages 31 to 40, and in later transfers, *i.e.* 50 to 58, not here shown, the per cent on the 4th day approached 90. The total number of reactors and the average length of the incubation period are demonstrated in Table III.

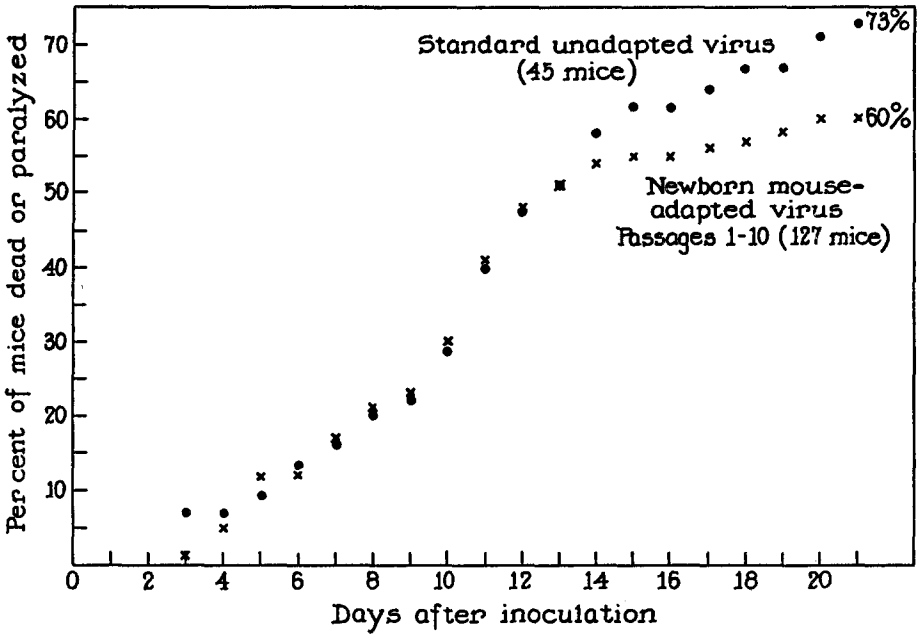

FIG. 2. Standard, unadapted virus compared with newborn mouse-adapted virus (passages 1–10) in 3- or 4-day-old mice. Cumulative percentage of mice paralyzed or dead.

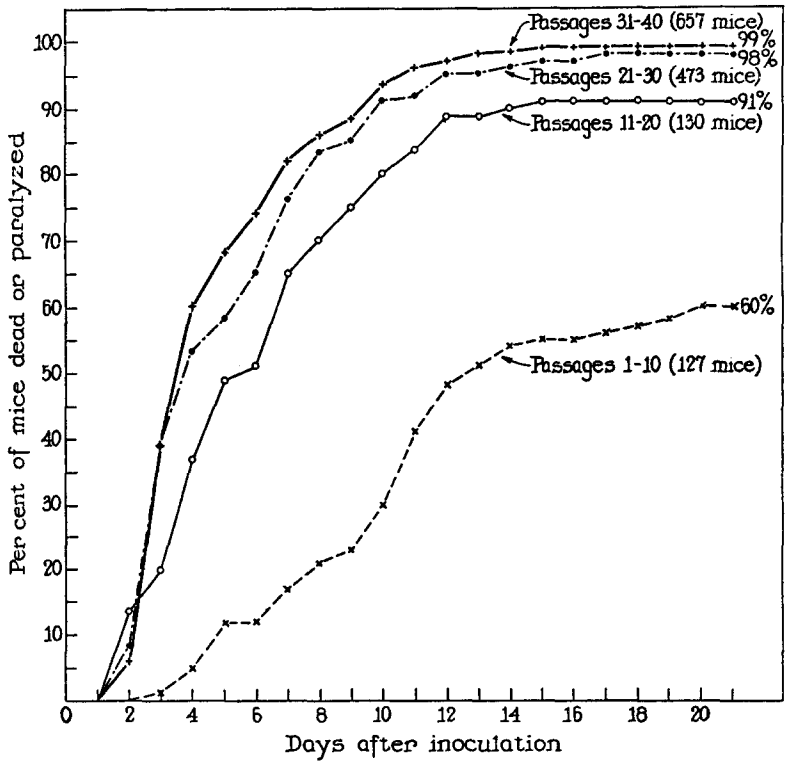

FIG. 3. MEF1 poliomyelitis virus adapted to newborn mice. Intracerebral inoculation of 10^{-1} dilution in 3- or 4-day-old mice. Cumulative percentage of mice paralyzed or dead.

Apart from the uniformly short and regular incubation period following intracerebral inoculation, and its use as a complement-fixing antigen (13), the adapted virus produced an increase in titer, particularly when tested in newborn mice.

Titer of the Adapted Virus Tested in Adult Mice.—The standard MEF1 strain was titrated intracerebrally in adult mice, *i.e.* 21 days old or older, and 30 such titrations carried out over a period of several years gave a geometric mean of $10^{-3.50 \pm 0.447}$, with limits of $10^{-2.60}$ and $10^{-4.50}$. Titrations of the adapted virus performed in the same sort of adult mouse, were made at irregular intervals

TABLE III

Inoculation of Standard MEF1 Virus and Newborn Mouse-Adapted Strains, into 3- and 4-Day-Old Mice

Strain	Passages	No. of mice	No. dead or paralyzed	Incubation period
				days
Standard		45	34	11.7
Newborn mouse-adapted	1–10	127	77	10.1
	11–20	130	118	6.1
	21–30	473	465	5.5
	31–40	657	650	5.0
	47–50	278	278	3.3

during the course of the transfers here described, with the following results:–

Passage 13, $10^{-3.9}$	Passage 32, $10^{-3.8}$
" 15, $10^{-4.6}$	" 36, $10^{-4.0}$
" 23, $10^{-4.5}$	" 38, $10^{-4.0}$
" 28, $10^{-4.8}$	" 53, $10^{-4.0}$

The geometric mean = $10^{-4.20 \pm 0.374}$.

Thus in titrations of MEF1-adapted virus by means of the intracerebral test in adult mice, no significant trend could be observed from passage 13 to 53. There was found, however, a slight increase in its titer over that of the standard strain, also tested in adult mice (adapted virus, $10^{-4.20 \pm 0.374}$ compared with standard strain, $10^{-3.50 \pm 0.447}$).

Titer of the Adapted Virus Tested in Newborn Mice.—In sharp contrast with the findings described in the preceding section are the results of titrations of the adapted virus performed in newborn mice.

The LD_{50} of the standard, Armstrong virus as determined by the intracerebral test in newborn mice was usually from 10^{-1} to 10^{-2} (Fig. 1). The standard MEF1 strain, similarly titrated in 1-, 3-, and 4-day-old mice (Table IV) yielded practically the same values. On the other hand, the titers of the adapted MEF1 strain in its 36th to 53rd passages in newborn mice and tested also in

TABLE IV

Titrations in Newborn Mice
Standard and Newborn Mouse-Adapted Strains of MEF1 Virus

Strain	Experiment	Age	Log dilution of virus					LD_{50}
			−1	−2	−3	−4	−5	
		days						
Standard	1	1	11/18*	3/15				1.32
		4	8/10	5/11	3/10	0/8		2.00
	2	1	9/11	6/12	0/8	0/6		1.84
		3	6/9	2/10	2/8	0/8		1.54
	3	1	14/17	7/14	0/13	0/8		1.19
		3	12/15	14/15	2/13	0/10		2.56
Newborn mouse-adapted	4	1	10/10	5/10	3/9	0/8		2.26
	P 36‡	3	9/9	3/8	1/7	0/7		1.90
	5	1	7/7	16/16	6/8	2/8		3.50
	P 38	3	7/7	10/10	6/10	3/9		3.43
	6							
	P 53	3	9/9	7/7	6/9	3/7	0/6	3.56

* 11/18 = 11 mice were paralyzed or died of 18 inoculated.
‡ P = newborn mouse passage.

TABLE V

Titration of MEF1 Poliomyelitis Virus Intracerebrally in Mice 1, 3, and 35 Days Old

Age of mice	Standard adult mouse strain						Newborn mouse-adapted strain					
	Log dilution of virus					LD_{50}	Log dilution of virus					LD_{50}
	−1	−2	−3	−4	−5		−1	−2	−3	−4	−5	
days												
1	9/11*	6/12	0/8	0/6		1.84	7/7	16/16	6/8	2/8		3.50
3	6/9	2/10	2/8	0/8		1.54	7/7	10/10	6/10	3/9		3.43
35	8/8	8/8	7/7	2/6	1/8	3.88	8/8	8/8	7/8	2/8	5/8	4.00

* 9/11 = 9 mice died of specific infection of 11 inoculated.

1-, 3-, and 4-day-old animals were definitely higher than those given by the standard virus.

The increased pathogenicity of the adapted strain for newborn mice is again noted in Table V in which is also given a comparison of the LD_{50} titers of the standard MEF1 strain and its newborn mouse-adapted form. When the

standard virus was titrated by intracerebral injection into newborn and adult mice, the latter yielded LD_{50} readings 2 and 2.3 log units higher than the titers in the newborn, whereas with the adapted form of the same strain, adults exhibited on an intracerebral test only 0.5 and 0.6 log unit higher infectivity titers than the newborn animals.

Distribution of the Adapted Virus in the CNS of Newborn Mice.—The infectivity titer was now investigated of the adapted strain in different parts of the CNS after its introduction into newborn mice, and at different times following injection.

TABLE VI

Distribution of Newborn Mouse-Adapted Virus in the CNS

Day after injection when killed	Part of CNS tested	Age of mice used in titration	Log dilution of virus*					LD_{50}
			−1	−2	−3	−4	−5	
		days						
2nd	Brain	28	5/6	4/6	5/6	4/6	2/6	4.00
		3	9/9	7/7	6/9	3/7	0/6	3.56
	Cord and brain stem	28	6/7	5/6	5/6	4/6	1/6	4.00
		3	9/9	7/7	6/9	0/7	1/8	3.33
4th	Brain	28	4/6	3/6	2/6	4/7	1/6	2.73
		3	9/9	6/8	6/8	3/8	1/9	3.53
	Cord and brain stem	28	6/6	4/6	6/6	4/6	2/6	4.25
		3	5/5	9/9	6/8	1/9	0/10	3.41

* 5/6 = 5 mice were paralyzed or died of 6 inoculated.

Six mice, 3 days old, intracerebrally injected with 10^{-1} dilution of adapted virus, passage 52, were killed at the onset of CNS signs, on the 2nd day after inoculation. The brain stems together with the spinal cords were harvested and pooled in one lot, and the remaining cerebral tissue in another. The two pools were then weighed, ground, suspended in diluent, and titrated in 3-day-old and in 28-day-old mice. A similar procedure was carried out with 6 additional mice from the same passage, which first became paralyzed on the 4th day after injection. The result of this experiment is shown in Table VI.

The infectivity titers of the adapted strain as tested in newborn and in adult mice were not markedly different. The LD_{50} titers, however, were somewhat lower in the former. Moreover, the titrations in adult mice revealed that on the 4th day after inoculation less virus was found in the brain than in the combined brain stem and spinal cord.

It would appear that in the early stages of the infection, on the 2nd day, the adapted virus was equally well distributed over the entire CNS, but that at a

later stage, on the 4th day, the brain stem and spinal cord contained more virus than the remaining cerebral tissue. This increase is demonstrable only by the use of adult mice for the titration of infectivity, these being more susceptible than newborn, as a rule, to Lansing-type poliomyelitis virus whether in its standard or adapted form.

DISCUSSION

Newborn mice are considerably more resistant to the Lansing-type poliomyelitis viruses than adult mice (10, 7, 12). The increased resistance is reflected mainly in the failure of many of them to react; as also in prolonged incubation periods of the reactors, and in lower infectivity titers as determined by the inoculation of newborn animals. The mechanism underlying the increased resistance is not known: in tests carried out in this laboratory no evidence could be obtained that it is based on the presence of natural, circulating virus-neutralizing antibody in the newborn, or of other substances readily extractable from their CNS tissues that might conceivably neutralize the virus.

One may assume that the serial passage in newborn mice which results in a strain of virus more actively pathogenic for them comes about because a suspension of the standard, unadapted virus contains a mixture of different kinds of viral particles. The majority of the particles are perhaps adapted to the CNS of adult mice but not to that of the newborn. Since the latter are not wholly resistant, the assumption just made would include the conception that a smaller number of viral particles in the inoculum are capable of attacking the CNS of the newborn as well as that of the adult. If this is so, then the rapid serial passage in the newborn results in a shifting of the virus population towards a larger proportion of particles pathogenic for newborn mice, thus leading to a definite adaptation of the virus to them, without loss of pathogenicity for the adult. The possible introduction of extraneous or contaminating viruses during the course of serial passage, that might have supplanted the original poliomyelitis strain, is excluded by the results of repeated checks, by means of immunological and serological tests, which have consistently indicated that there has been no loss of immunological or serological identity of the adapted strain with its parent.

CONCLUSION

By means of rapid serial passages, including 3 successive "blind" passages, the MEF1 strain, a Lansing-type poliomyelitis virus, has been adapted to newborn mice. The virus can readily be propagated in newborn mice, in which fully adapted virus induces in almost all inoculated animals the experimental disease, resulting in a much greater infectivity for the central nervous system and a uniformly short and regular incubation period.

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