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Intracerebral Adaptation to Mice of the Leon Strain of Type 3 Poliomyelitis Virus.* (20876)

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The adaptation to mice of the Leon Strain of type 3 poliomyelitis virus employing the intraspinal route has been reported by Li and Habel(1). Their results have been confirmed by Casals, Olitsky, and Brown(2). In a more recent paper Li and Schaeffer(3) have described a further modification of the mouse adapted line of the Leon Strain; continued mouse passage increased the virulence for mice but decreased intracerebral infectivity for the monkey. Both Li and Schaeffer(3) and Habel (ref. cited in 3) noted that this strain would occasionally infect mice by the intracerebral route. The present report gives details of procedures whereby this virus has been adapted to mice resulting in a line that is pathogenic via the intacerebral route as well as the intraspinal.

Methods and materials. Technic of criss-cross passage. In the adaptation experiments to be described criss-cross passages were used between tissue cultures and mice, injecting tissue culture fluid intracerebrally in the mouse and recovering, in tissue cultures, the virus from the cords of animals thus paralysed. The 2nd tissue culture passage was again inoculated intracerebrally and the criss-cross passages repeated in the same way. The tissue culture fluids of various passages were then titrated in mice by the intracerebral and intraspinal routes. *Viruses.* Drs. Li and Schaeffer, to whom we express our thanks, were kind enough to send to us their line of the Leon Strain, adapted for intraspinal infection of mice. The material consisted of 10% cord suspension and was taken from the 81st mouse passage. During our experiments, this strain was typed frequently in tissue cultures and identified as type 3 poliomyelitis virus. A second strain (Lansing, type 2), which is well adapted for the intracerebral route in mice, was used for comparative titration both intraspinally and intracerebrally in mice. *Test in mice.* CFW mice (15-20 g) were used throughout these experiments and a standard inoculum of 0.025 ml was employed for both the intracerebral and intraspinal route. The technic of the intraspinal inoculation was the same as described by Habel and Li(4). Average loss of mice paralysed by trauma was about 10% in the present studies. Animals found dead or paralysed after 12 to 24 hours were eliminated from the final results. Only cords of animals found paralysed after cerebral inoculation were pooled and used as 10% tissue suspension for inoculation into tissue cultures. It was felt that the ability to invade the cord might be one characteristic of a pathogenic virus. The cord suspension was prepared in distilled water and clarified with streptomycin according to a

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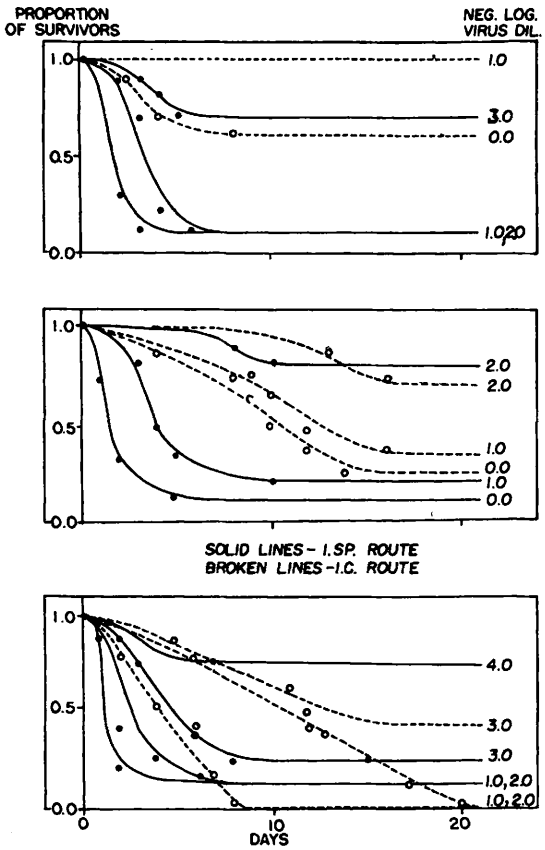

Figure 1 A
Leon 1 st. criss-cross passage

Figure 1 B
Leon 10th criss-cross passage

Figure 1 C
Lansing Pool
No. 062652

FIG. 1. Response time distribution for intracerebral and intraspinal route after the first and tenth criss-cross passage of mouse-adapted Leon virus as compared with the Lansing virus.

method developed by B. L. Bennett(5). *Tissue cultures.* We inoculated the Leon Strain into tissue cultures which were prepared according to a method developed by Dulbecco and Vogt(6). Cynomolgus monkey kidney mince was treated with 0.25% trypsin. The cell sediment was suspended in Synthetic Mixture 199 (Morgan, Morton and Parker) (7), giving a final concentration of cell sediment of 1/200. Two percent inactivated horse serum, 0.08% $NaHCO_3$ and penicillin and streptomycin were added to a final concentration of 100 units and 0.1 mg per ml respectively. The cultures in "milk-dilution bottles" were incubated at 36°C in a horizontal position and without motion for 6 days with a medium change after the 3rd day. Usually a complete tissue sheet covered the bottom of the bottles after 6 days. Before the inoculation of the virus the medium was changed again, but no horse serum was added. The final dilution of the virus in the inoculated vessel was $10^{-2.5}$. The cultures were kept at 36°C and harvested as soon as all tissue was degenerated. This degeneration usually occurred between the 6th and 8th day. By this method, fluids with high infectious titers could be obtained in sufficient quantities. It might be mentioned at this point that the mouse adapted Leon Strain behaved somewhat differently in tissue cultures when compared with certain other strains of each of the three types that were well established in this medium. The latter strains produced complete tissue degeneration between the 2nd and 4th day; whereas the mouse adapted Leon Strain showed a longer incubation period of 6-8 days. It is of further interest that undiluted supernatant fluid from a 10% cord suspension from Leon paralysed mice was

TABLE I. Proportion Responding (Paralysis or Death) for Both the Intracerebral and Intraspinal Route of the Leon Strain after Various Criss-Cross Passages. Adapted Lansing strain for comparison.

Strain	Passage	Negative logarithms of virus dilutions inoculated: Intracerebrally					Intraspinally				
		0	1	2	3	4	0	1	2	3	4
Leon (Type 3)	1	4/10	0/10	0/10	0/10	—	9/10	9/10	3/10	0/10	—
	3	9/10	1/10	0/10	0/10	—	9/10	10/10	10/10	3/10	—
	8	10/10	10/10	2/10	0/10	—	10/10	9/10	6/10	0/10	—
	10	7/ 8	6/ 8	2/ 8	0/ 8	—	9/10	8/10	2/10	1/10	—
	13	10/10	10/10	9/10	6/10	—	11/11	11/11	10/10	9/10	1/10
Lansing (Type 2)		—	8/ 8	8/ 8	5/ 8	0/8	—	7/ 8	7/ 8	6/ 8	2/ 8

inoculated into tissue culture and produced no cytopathogenic effect over an observation period of 10 days.

Results. Fig. 1 shows the proportion of survivors when plotted against time in various titrations by the intraspinal as well as by the intracerebral route for type 2 and 3 poliomyelitis virus. Similar results were obtained when these fluids were titrated on different occasions, and this Figure illustrates the results of a typical experiment. A comparison of Fig. 1,A and 1.B shows clearly the shift of the response-time distribution of the intracerebral route towards that of the intraspinal one; this occurred between the 1st and 10th criss-cross passages. Thus this line of the Leon Strain is beginning to behave like the well adapted Lansing Strain (Fig. 1,C). It is of interest to note in Fig. 1,A the short incubation periods in the responding animals with early truncation of the curve for the intracerebral inoculated animals. This observation was consistent for material from the early passages. The behavior of the later passages is as shown in Fig. 1,B.

The proportion of responding animals for various virus dilutions tested is recorded in Table I, which shows another measure of adaptation. A rough estimate gives a potency† of intracerebral as compared with intraspinal route in the first passage of 0.01 while in the 10th passage it is about 1; correspondingly, the intracerebrally adapted Lansing Strain shows a relative potency of 1. A marked change of the relative potency occurred somewhere between the 3rd and 8th criss-cross passage.

Summary. The intracerebral adaptation of the Leon Strain of type 3 poliomyelitis to mice has been described.

† Relative potency, as commonly defined in bioassay, is the ratio of equally effective doses. Its logarithm may be taken as a difference between negative logarithms of two RD_{50} values, where RD_{50} is defined as the dilution estimated to give a 50% response (paralysis or death).

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