toneal injection of undiluted egg-white leads to a *maximal* increase in capillary permeability in the paws, and that, in this situation, the protective effect of certain inflammatory substances used for pre-irritation might become less evident.

Summary. 1. Topical protection against hyperergic inflammation by a preceding local inflammation, and topical protection against thermal injury by a preceding local thermal irritation can be demonstrated even in rats adrenalectomized before pre-irritation. 2. Acquired topical protection, therefore, is not likely to depend upon the topical extravasation of antiphlogistic corticosteroids into pre-inflamed areas.

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Received March 29, 1954. P.S.E.B.M., 1954, v86.

Susceptibility of Mice to Infection with the Mahoney Strain of Type 1 Poliomyelitis Virus.* (21047)

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A line of the Mahoney strain Type 1 poliomyelitis virus has been adapted by Li and Schaeffer(1) to produce intraspinal infection in mice. This strain has been used to demonstrate neutralizing antibodies in serum specimens(2), and also to demonstrate resistance upon challenge of mice immunized with Mahoney virus(3). Since the intraspinal inoculation is a rather traumatizing procedure and the non-specific response due to trauma, depending on the skill of the operator, is considerable, a line adapted to the intracerebral route might therefore be advantageous. Furthermore, increasing adaptation might eventually result in a line pathogenic to mice following peripheral inoculation.

It is the purpose of this report to give details of the procedure that resulted in a line of Mahoney virus pathogenic via the intracerebral route. This strain will also induce paralysis in a small proportion of mice following intravenous inoculation.

Materials and methods. Virus. Mahoney strain virus adapted for intraspinal infection of mice was kindly provided by Drs. Li and Schaeffer as 10% cord suspension representative of the 35th mouse passage. This material was used for intraspinal titration in mice and as the inoculum for tissue cultures. Four-week-old Swiss albino mice, CFW strain, were employed as routine. Tissue culture fluids from criss-cross passages 3, 4, 6 and 7 were identified as Type 1 poliomyelitis virus: a) by neutralization test in tissue cultures using homotypic antibody for poliomyelitis types,

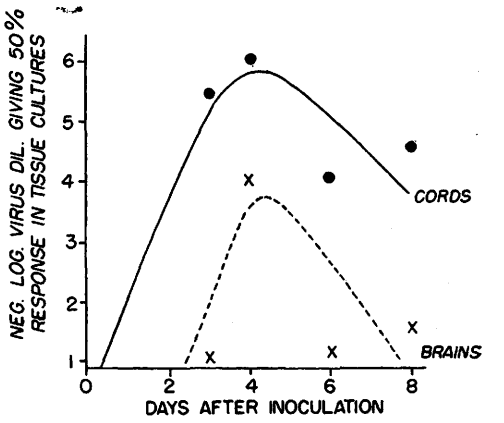

FIG. 1. Distribution of virus in brains and cords of paralyzed mice at various intervals after intravenous inoculation of the 4th criss-cross passage fluid.

* Aided by a grant from The National Foundation for Infantile Paralysis.

1-2-3; b) by intracerebral challenge of mice immunized by inactivated Type 1 virus. The technic for adaptation consisted of serial criss-cross passages between tissue cultures and mice by the injection intracerebrally, or intravenously of tissue culture fluids into mice and by recovery in tissue culture of virus from cord tissue of paralyzed animals. The technics for criss-cross passage, the preparation of tissue cultures, and the preparation of the cord suspensions were described previously (4).

Undiluted tissue culture fluid from the first passage was tested in mice by injection intracerebrally, 0.03 ml, or intravenously, 0.5 ml. For successive passages, the inoculum consisted of cord from the first mouse paralyzed from intracerebral injection when intravenous injection had proved ineffective within 5 days from injection. Tissue culture fluid from the 4th criss-cross passage was used as inoculum to study the distribution of virus in brain and cord of mice developing paralysis following intravenous inoculation. Tissue suspensions of cord and brain from paralyzed mice were prepared and the supernatant fluid after centrifugation was titrated in 10-fold dilution steps for virus activity in tissue cultures using 3 cultures per level. Mice receiving the unadapted Mahoney strain intravenously did not develop paralysis. Their cords and brains were treated in the same manner and used for comparison.

Results. The titer of virus in the cord was more than 100-fold higher than in the brain of the same animals. The highest virus concentration in the cord was found in mice paralyzed early after inoculation, the titer was less in the 6- and 8-day specimens. These findings are in good agreement with those of Herrarte and Pearson(5) who followed the virus concentration in the cords and brains of mice after intracerebral inoculation with Type 2 poliomyelitis virus. No virus was recovered from mice inoculated with the unadapted Mahoney virus.

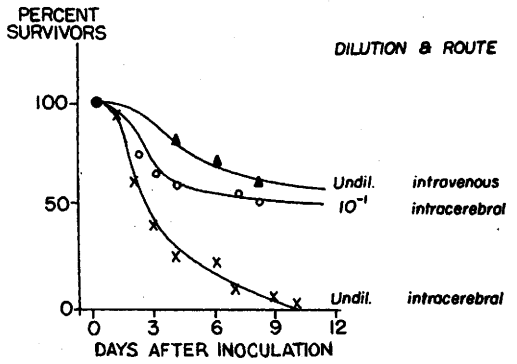

FIG. 2. Response-time relationship in mice inoculated with tissue culture fluid from the 7th criss-cross passage.

Fig. 2 shows the time-response relationship for the intracerebral and intravenous inoculation of tissue culture fluid taken from the 7th criss-cross passage. Twenty mice were used for each 10-fold dilution. 50% survival time estimated from this graph is about 2.5 days for the group inoculated intracerebrally with undiluted tissue culture fluid, the incubation period is 2 to 6 days, sometimes as short as one day but rarely animals responded later than the 10th day.

TABLE I. Potency of Tissue Culture Fluids Harvested from Various Criss-Cross Passages Tested in Tissue Cultures and in Mice Inoculated by Different Routes.

	Tissue cultures				Mice								
					Intraspinal					Intracerebral			Intravenous
Doses (ml)	.25				.025					.025			.5
Neg log dilutions	5	6	7	Log ID_{50}/ml	2	3	4	5	Log ID_{50}/ml	0	1	Log ID_{50}/ml	0
35th M.P.					5/8	4/8	0/8	0/8	4.1				
1st C.C.P.	3/5	0/5	0/5	5.6	5/10	1/10	1/10	0/10	3.6	1/10	0/10		0/10
2nd	4/5	1/5	0/5	6.0						5/20	0/20	0.75	
3rd	2/5	1/5	0/5	5.6	9/10	6/10	1/10	0/10	4.5	9/20	1/20	1.5	5/12
4th	4/5	3/5	0/5	6.5						15/20	4/20	2.0	4/20
5th	5/5	3/5	1/5	6.8	10/10	5/10	1/10	0/10	4.6	12/20	4/20	1.75	3/20
6th	5/5	1/5	3/5	6.8	10/10	7/10	3/10	0/10	5.0	16/20	6/20	2.0	2/20
7th	5/5	5/5	3/5	7.5	10/10	10/10	4/10	2/10	5.5	20/20	8/20	2.3	8/20
8th	5/5	5/5	5/5	>7.5						20/20	12/20	2.5	

TABLE II. Neutralization Test of Type 1 Passage Virus in Mice and Tissue Cultures. Entries Are Proportions Responding.

Neutralization test in	Virus passage	Approx. ID_{50}	Immune monkey serum			Normal monkey serum
			Type 1 Brunhilde	Type 2 Lansing	Type 3 Saukett	
Mice (I.C.)	7th C.C.P.	32	2/20	18/20	20/20	20/20
Tissue culture	" "	1000	0/3	3/3	3/3	3/3
		100	0/3	3/3	3/3	3/3

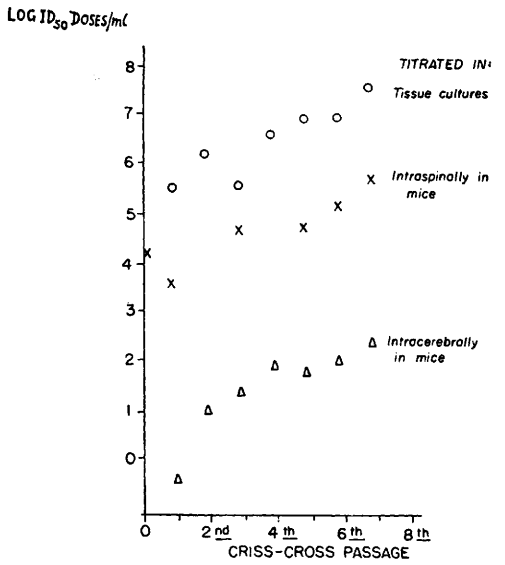

FIG. 3. The potency at various passage levels of the Mahoney strain in tissue cultures, and in mice inoculated by different routes.

Table I contains data on the potency of tissue culture fluids from the 1st to the 8th criss-cross passage when tested in mice inoculated by various routes as well as in tissue cultures. The ID_{50}/ml taken from this table are plotted in Fig. 3. These data suggest that during the course of serial criss-cross passages the potency of the tissue culture fluids increased for mice as well as for tissue cultures.

Evidence that this strain retained its immunological identity during the course of these adaptation experiments is presented in Table II. The cytopathogenic effect in tissue cultures and the infectivity in mice is neutralized by an anti-Brunhilde monkey immune serum. Equally potent immune sera representing Type 2 and 3 poliomyelitis virus as well as normal monkey serum had no neutralizing effect.

Discussion. Evidence is presented for the successful adaptation of the Mahoney strain to the intracerebral route in mice. The same material induces paralysis in a small proportion of mice following intravenous injection. The rise in infectivity following criss-cross passages is suggested by the data. However, it is questionable whether this is the only factor involved in the adaptation. So far only 2 out of 6 Type 1 fluids having the same potency for tissue cultures as the 6th criss-cross passage have induced paralysis in a small number of mice inoculated intraspinally.

In the course of these experiments the strain under investigation acquired the ability to invade the spinal cord, and high concentration of virus in the cord is associated with the development of paralysis. The dissociation in infectivity for the intraspinal and intracerebral route of inoculation is striking. A well adapted Lansing strain will be almost equally potent by those two routes. Therefore, it seems conceivable that the Mahoney strain is not yet adapted to its full mouse pathogenicity or, in this respect, may behave differently from the Lansing strain.

Summary. Criss-cross passages between mice and tissue cultures resulted in the adaptation of Type 1 (Mahoney) poliomyelitis virus to the intracerebral route in mice.

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Received April 9, 1954. P.S.E.B.M., 1954, v86.