Infection of Mice with Types I and III Poliomyelitis Virus Following Intracerebral Inoculation. (20915)

N. F. STANLEY, D. C. DORMAN, AND JOAN PONSFORD. (Introduced by G. Dalldorf.)

From the Institute of Epidemiology and Preventive Medicine at The Prince Henry Hospital, Sydney, N. S. W.

Li and Habel(1) recently described the adaptation of type III (Leon strain) poliomyelitis to mice, following intraspinal inoculation. Subsequently Li and Schaeffer(2) reported the successful adaptation of type I (Mahoney strain) poliomyelitis virus to mice employing a similar technic. They reported that rodents could be successfully infected only by the intraspinal route and failed to infect animals by the intracerebral route. The same authors claim in a more recent paper(3), that with the Leon strain they "can now occasionally infect mice by the intracerebral route."

It is the purpose of this communication to describe the results of further passage of these mouse-adapted strains in an Australian mouse stock using the intracerebral route of inoculation.

Materials and methods. Virus strains. Through the courtesy of Dr. Schaeffer, the following strains were forwarded to this laboratory—2 mouse cords infected with type I poliomyelitis (Mahoney strain) after 44 passages by the intraspinal route and 2 mouse cords infected with type III poliomyelitis (Leon strain) following 97 passages in mice by the intraspinal route. *Animals.* All mice used in these experiments came from this laboratory's animal house and are referred to as The Prince Henry mice. These mice have been brother to sister bred in a large isolation unit for 13 years without the introduction of any outside stock. They originally derived from the mice used by Robertson(4). A latent Theiler's encephalomyelitis virus has not been demonstrated in this stock, although repeated attempts have been made to do this since 1948. The monkeys used in these experiments were imported from Singapore and comprised the species *M. irus* and *M. nemestrinus*. All had been castrated from some weeks to several months prior to inoculation, the testes being used for tissue culture. *Inoculations.* Intraspinal (I.S.) inoculations were carried out as described by Habel and Li(5). Four-weeks old mice were used for both I.S. and intracerebral (I.C.) inoculations. With the latter route, the mice were lightly anesthetized, and the hair of the head swabbed with 70% ethanol. The I.C. inoculum consisted of 0.04 ml delivered from a tuberculin syringe. Monkeys received 0.3 ml in the left thalamus. *Preparation of virus suspensions.* Suspensions of infected mouse cords were made as follows: Paralyzed mice were killed with diethyl ether. The brains were removed and the vertebral column cut with scissors immediately above the sacrum. An 18-gauge needle attached to a syringe containing sterile normal saline was firmly inserted in the spinal canal and pressure applied to the plunger, thus forcing the cords out through the foramen magnum. The cords were ground (without abrasive) in chilled mortars and a 10% suspension made in chilled ¼ strength broth. The suspension was cleared by centrifuging at 1500 rpm for 5 minutes in a refrigerated International centrifuge. A penicillin-streptomycin mixture was added to give a final concentration of 100 units of penicillin and 50 units of streptomycin per ml and the suspension used immediately for animal inoculations. *Neutralization tests.* To the test serum (inactivated at 55°C for 30 minutes) was added an equal volume of 10% infected cord suspension (passage 12). The serum-virus mixture was allowed to stand at room temperature for one hour before I.C. inoculation of the mice. *Tissue culture.* This was carried out using monkey testis grown in bovine amniotic fluid(6) and was a modification of the method described by Enders(7). *Sera.* In the neutralization tests carried out in mice by the I.C. route, the following sera were used. (a) Serum from a poliomyelitis patient who subsequently died from the disease. This serum contained tissue culture neutralizing

antibodies against the Mahoney (type I) strain to a titer >1/160, but no detectable antibody to either the Leon or MEF1 strains. (b) Hyperimmune mouse serum against the MEF1 strain of poliomyelitis. This serum contained specific neutralizing antibodies to a titer of 1/160 (tissue culture). (c) Leon hyperimmune monkey serum containing specific neutralizing antibodies to a titer >1/160 (tissue culture).

Experimental. Type I poliomyelitis (Mahoney strain). Contrary to the experience of Li and Schaeffer, this strain could not be successfully passaged in The Prince Henry mice by the I.S. route. I.C. inoculations, however, presented quite a different picture. The primary inoculation of type I infected mouse cords (ex U.S.A. passage 44) resulted in 4 of 8 mice becoming paralyzed following I.C. inoculations and one of 8 mice becoming paralyzed following I.S. inoculation. Virus could not be demonstrated by further I.C. or I.S. passage of the cord from the I.S. line. On the other hand the cords from the I.C. inoculated mice caused paralysis of passage one mice only by the I.C. route and not by the I.S. route. At this stage virus could not be detected in the brains of paralyzed mice but only in the cords. Accordingly, from passage 2 material onwards the *cords* of paralyzed mice were passaged by the I.C. route. It was decided to use 20 mice for each passage as not all animals inoculated became paralyzed.

TABLE I. Results of I.C. Inoculation of 322 Mice with Mahoney Virus during the First 12 Passages in Prince Henry Mice.

Passage No.	No. mice inoc.	No. of mice paralyzed — Days after inoc. 2	3	4	5	6	7	8	Total
Primary	8			4					4
1	8		1	1					2
2	8		3						3
3	20		5	1	1	1		1	9
4	20		3	1					4
5	80	5	20	14	3	2			44
6	20	6	2	1	1		1		11
7	20	2	6						8
8	20	2	8						10
9	19			3					3
10	20		5	3		1	1		10
11	19	4	3						7
12	60	10	28	5					43
Total	322	29	84	33	5	4	2	1	158

TABLE II. Neutralization Tests with the Modified Mouse-Adapted Type I (Mahoney Strain) Poliomyelitis Virus Using the I.C. Route of Inoculation in 4-Week-Old Mice.

Serum containing antibody to type I (human)	II (mouse)	III (monkey)	Control
0 * / 19	14 / 20	14 / 20	13 / 20

* Numerator = No. of mice paralyzed.
Denominator = No. of mice inoculated.

The results of the first passages may be briefly summarized. Only 34% of the animals inoculated during the first 5 passages became paralyzed. From passages 6 to 12, 52% of the animals became paralyzed. Of the paralyzed animals, 70% showed paralysis by the third day after inoculation and 92% by the fourth day. Table I shows the results obtained from an examination of the first 12 passages during which 322 mice were inoculated and 158 became paralyzed.

One interesting feature of the paralysis was that with 2 exceptions paralysis was observed in the fore-limbs and not in the hind-limbs. The impression gained was that the virus was in higher concentration in the cervico-thoracic area of the cord than in the lumbar area. On several occasions various sections of the cord were passaged. With a lumbar-cord suspension only one of 20 mice inoculated became paralyzed, and then only in the hind limbs.

Infectivity for monkeys. Four monkeys were inoculated intracerebrally with passage 8 cord suspension. These animals remained asymptomatic for a period of two months after inoculation.

Neutralization tests. The antigenic character of the infective agent has been examined only by neutralization tests which were carried out with the infected cords from passage 12. The results are shown in Table II.

Type III poliomyelitis (Leon strain). The sequence of events following inoculation of type III infected mouse cords (ex U.S.A. passage 97) into Prince Henry mice by the I.S. and I.C. routes, duplicates in many ways the results obtained with the type I mouse adapted poliomyelitis virus. The results of the primary inoculation and the first 5 passages only will be presented. Virus could be

TABLE III. Results of I.C. Inoculation of 92 Mice with Leon Virus during First 5 Passages in Prince Henry Mice.

Passage No.	No. mice inoc.	No. of mice paralyzed — Days after inoc. 2	3	4	5	6	7	8	9	10	Total
Primary	8				2						2
1	8			2							2
2	20			1	1	2				1	5
3	20				2		1				3
4	20	1		2	2		2				7
5	16		1	1	2	1					5
Total	92	1	1	6	9	3	3	0	0	1	24

demonstrated by the I.C. route but not by I.S. inoculation. During these early passages 26% of mice inoculated became paralyzed compared with 34% to type I virus. Again only the fore-limbs were affected. The average incubation period was longer, the majority of animals becoming paralyzed on the 4th and 5th days after inoculation (Table III). The impression gained was that The Prince Henry mice were not as susceptible to infection with type III as with type I mouse-adapted virus. Neutralization tests have not yet been performed to confirm the identity of this strain.

Discussion. There are several interesting points arising from these preliminary experiments. Probably the most important is the observation that type I poliomyelitis virus will infect mice when given by the intracerebral route. In passaging this virus, mouse cord (and not mouse brain) was inoculated intracerebrally, as virus was found in higher concentration in the cord. The cord, therefore, contained virus which was capable of *invading* C.N.S. tissue of mice.

The ability to infect mice by the I.C. route and not by the I.S. route differs from the findings of Li and Schaeffer(2), who kindly supplied the authors with the strains adapted to mice by I.S. inoculations. At this stage there is little evidence to indicate why this difference should exist. It is considered likely that the genetic constitution of The Prince Henry mice is different from the stock used in Alabama. The short incubation period and the ease with which I.C. inoculations could be performed compared with I.S. inoculations, suggest that these observations may be of some help in laboratory studies of poliomyelitis.

Summary. The type I and type III strains of poliomyelitis adapted to mice by the intraspinal route by Li and Habel(1), and Li and Schaeffer(2) were found to be infective by the *intracerebral* route for The Prince Henry mice in Australia. Examination of the behavior of the type I strain during the first 12 passages in Prince Henry mice revealed that there was a slight increase in virulence resulting in more than 50% of the animals becoming paralyzed with an average incubation period of 3 days. Nearly every infected mouse was paralyzed in the fore-limbs only. The virus was found in highest concentration in the cervico-thoracic area of the cord, but was demonstrated in lower concentration in lumbar cord and brain. Monkeys remained asymptomatic following intracerebral inoculation with this strain.

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Received February 23, 1954. P.S.E.B.M., 1954, v85.