but the evidence from *Paramecium* does not rule out other interpretations; *e.g.*, an effect of oxygen itself upon the recombination of broken chromosomes.

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Adaptation of Type I Poliomyelitis Virus to Mice. (20151)

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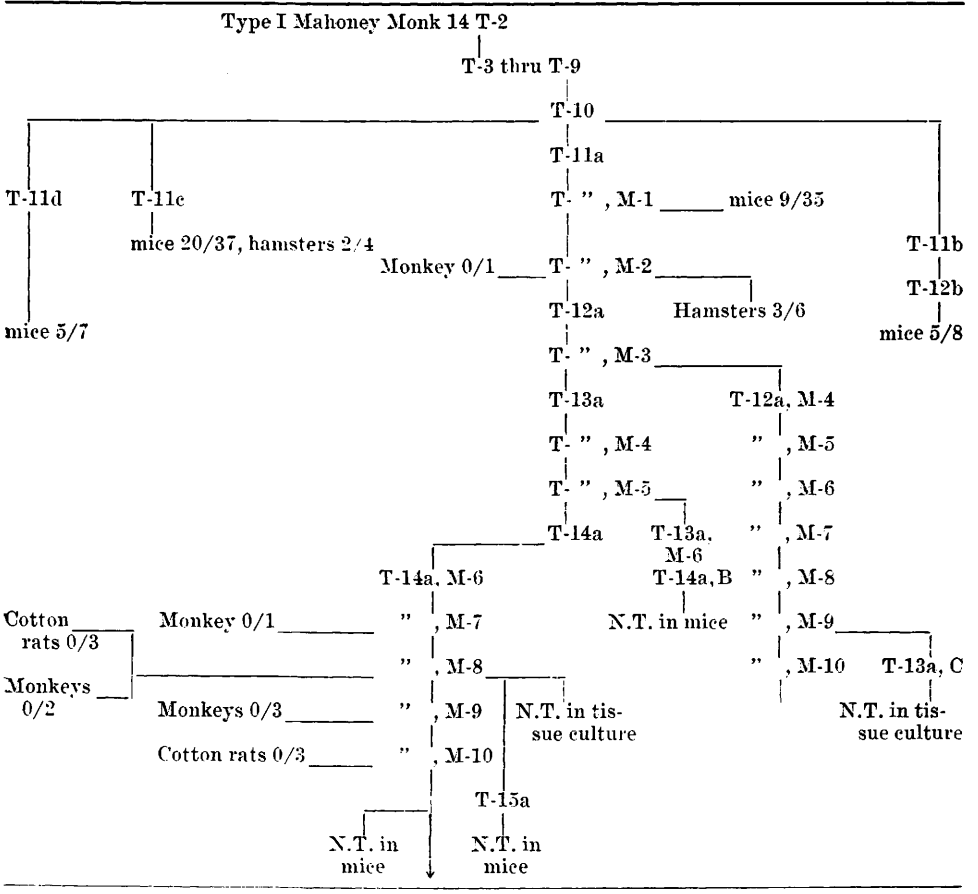
Since the adaptation of the Type II (Lansing) strain of poliomyelitis virus to mice by Armstrong(1), numerous attempts have been made to accomplish this with other poliomyelitis virus strains. Success with the Type III (Leon) strain was reported by Li and Habel (2), employing the intraspinal route in mice (3). This has been confirmed by Casals *et al.*(4), but the low virulence of the adapted strain has limited its usefulness in laboratory and field studies. More recently, Li and Schaeffer(5) have been able to increase the adaptability of the Type III strain to a degree of virulence which enhances its satisfactory utilization in mouse neutralization tests for the detection of antibodies.

Although Steigman and Kokko(6) have shown Type I poliomyelitis virus to persist in the mouse brain, they have not demonstrated that multiplication of the virus takes place. This report will describe methods employed in the successful adaptation of the Type I (Mahoney) strain to mice and its propagation in the rodent host.

Materials and methods. Virus. The Mahoney strain of poliomyelitis was employed in this work. This virus was originally isolated by Dr. Thomas Francis, Jr., from a pool of 3 stools of asymptomatic cases of poliomyelitis in Cleveland in 1941 and was later classified as Type I. The virus, when furnished us by Dr. Jonas E. Salk, had been through a total of 14 monkey passages and 2 monkey testicular tissue culture passages. *Immune serum.* Hyperimmune serum was prepared in monkeys with adjuvants following the procedure of Salk *et al.*(7) against Type I (Brunhilde), Type II (Lansing), and Type III (Leon) monkey propagated stock viruses. These sera were checked for specificity both in mouse and tissue culture neutralization tests. Immune monkey sera prepared and tested against the 3 types of virus in other poliomyelitis laboratories were obtained and also were used in neutralization tests to prove the specificity of the adapted virus. *Animals.* In nearly all the experiments performed, CFW mice were used. These were 3- to 5-week-old mice in the earlier passages, but later 8- to 12-week-old mice were employed because of preliminary indications that the latter may be more susceptible. Hamsters, cotton rats, and C3H mice were tested on 2 occasions. When monkey infectivity tests were conducted, rhesus monkeys weighing 3 to 6 pounds were inoculated intracerebrally. The rodents were successfully infected only by the intraspinal route. Intracerebral passages failed, and combined intraspinal and intracerebral inoculations were not superior. *Tissue culture.* The technic employed in our tissue culture has been described previously(8). Essentially, it

CHART I. Lines of Passage in Tissue Culture and Mice.



T = tissue culture passage; M = mouse passage; N.T. = neutralization test. Fraction = No. paralyzed/No. inoculated animals named.

is similar to methods currently utilized in most laboratories. Fragments of monkey testicular tissue are embedded in plasma clots on test tube walls, immersed in fluid consisting of one part of 5% bovine plasma hydrolysate and 3 parts of Hanks-Simms solution. The tubes are incubated in a slanted stationary position for several days to permit cellular outgrowth. Virus was cultivated in such cultures for 9 to 15 days, after which the tissue fragments in each tube were detached from the tube wall, ground, and suspended in about 0.3 ml of native culture fluid. The suspension was then lightly centrifuged and the supernatant fluid was used for animal inoculation. For serial virus tissue culture passages, only the fluid in amounts of 0.1 ml was transferred to successive tubes. *Neutralization tests.* These were conducted during the course of the work to test the specificity of the virus. Equal portions of heat-inactivated, undiluted immune serum were mixed with virus contained in 10 or 20% mouse cord suspension or in tissue culture fragments ground and suspended in their own fluids. The mixtures were incubated at 37°C for one hour, and 0.15 ml of each was inoculated into sets of tissue culture test tubes in triplicate, or 0.025 ml was inoculated intraspinally into groups of 8 mice for each mixture. Mice were observed for evidence of paralysis for a minimum of 2 weeks and tissue cultures for cytopathogenic changes for 6 days.

Experimental. As mentioned, the Type I virus with which these studies were begun had previously had 14 monkey passages fol-

TABLE I. Direct Passage of Type I Virus in Mice after Second Alternating Tissue Culture Passage.

Passage	Paralysis	Incubation period
T-12a, M-3	5/10*	3^3, 4^1, 9^1†
4	15/19	3^2, 4^2, 5^6, 6^1, 7^1, 8^1, 10^1, 12^1
5	5/13	4^2, 6^2, 16^1
6	4/12	3^1, 4^2, 17^1
7	4/12	4^2, 5^1, 10^1
8	12/19	2^1, 3^3, 4^3, 5^3, 6^3, 7^3
9	7/15	3^2, 5^3, 6^1, 7^1

* No. of mice paralyzed/No. inoculated.
† One mouse paralyzed on 9th day.

TABLE II.
Direct Passage of Type I Virus in Mice after Fourth Alternating Tissue Culture Passage.

Passage	Paralysis	Incubation period
T-14a, M-6	15/19*	3^{10},† 4^4, 7^1
7	16/20	2^1, 3^6, 4^5, 5^3, 7^1, 10^1
8	14/19	2^1, 3^9, 4^4
9	10/20	2^2, 3^4, 4^4
10	12/12	2^2, 3^9, 4^1

* No. of mice paralyzed/No. inoculated.
† Ten mice paralyzed on 3rd day.

lowed by 2 tissue culture passages. In this laboratory, it had 8 additional successive tissue culture passages. Material from these cultures up to the 4th passage failed to produce any apparent infection in mice when inoculated by various routes, including the intraspinal. The remaining 6 passages were made without further tests in mice. From the 10th passage, 4 lines of subpassages were started, each on a different occasion. Series T-11a consisted of alternation between tissue culture and mouse passages with periodic sublines of direct mouse-to-mouse passages. Series T-11b had only 2 additional tissue culture passages, while series T-11c and T-11d had but one. From each of these series, material when inoculated intraspinally into mice or hamsters was found capable of inducing paralytic infection in half, or more than half, of the injected animals.

It became apparent, therefore, that somewhere between the 5th and 11th tissue culture passage a rather striking variation in the behavior of the virus had occurred. This was manifested by its current ability to produce clinical disease in rodents following direct injection into the spinal cord, a characteristic not previously elicited by this technic.

Chart I depicts the lines of passage of the virus in tissue culture and mice, as carried out thus far. The material selected for infectivity and neutralization tests, and the results of some of these tests, are indicated on this chart. In the accompanying tables, there are presented in greater detail selected examples of the more pertinent data obtained in evidence of the selection of a rodent-adapted strain of Type I poliomyelitis virus.

Characteristics of the virus. Mice inoculated intraspinally with sufficient doses of the Type I modified virus develop typical poliomyelitic paralysis. The incubation period is 3 to 5 days, occasionally being as short as 2 days and rarely as long as 10 to 17 days. Paralysis usually appears first in one of the forelegs or hind legs and gradually spreads to all extremities. Some animals may survive in the paralytic condition for several days. In the early passages especially, paralysis was frequently mild and many of the mice recovered.

Virus derived from mouse cord suspensions had a greater infectivity titer for tissue cultures than for mice. In tissue cultures this virus was infective in titers of 10^{-4} to 10^{-5} while in mice infectivity ranged from $10^{-0.5}$ to 10^{-1}. However, when this virus was grown in tissue culture it not only retained the same tissue culture titer but also had a relatively higher (10^{-1} to 10^{-2}) mouse infectivity titer. With increasing numbers of mouse passages, direct or alternate, there appears to be a trend toward increasing virulence for mice (Table II), despite some fluctuation. In the first mouse passages only 9 of 35 mice were paralyzed, while in current passages the virus paralyzes 50 to 100% of the mice. With continued passage of this virus strain, the infectivity titers for mice of both the mouse-maintained and tissue-culture-maintained virus are gradually approaching each other.

The spinal cords of representative mice, occasionally sacrificed and autopsied following infection, when examined microscopically, have invariably revealed the typical histopathologic lesions of poliomyelitis. Small groups of monkeys were inoculated intracerebrally, mostly with late passage virus, but these remained asymptomatic. However, in one monkey, sacrificed 29 days after inocula-

TABLE III. Neutralization Test of Passage Type I Virus in Mice and in Tissue Culture.

N.T. in:	Virus passage	Material	Approx. I.D.	Immune monkey serum (Type) I	II	III	Normal monkey serum
Mice	T-14a, B	Tissue culture undil. susp.	100	0/8	6/8	8/8	7/8
				Cell degeneration in tubes			
Tissue culture	T-14a, M-8	Mouse cord 20% susp.	10000	1 2 3 — — —	1 2 3 + + +	1 2 3 + + +	1 2 3 + + +

TABLE IV. Specificity Tests on Control Monkey Immune Sera Tested against Heterologous Types of Poliomyelitis Viruses in Tissue Culture and in Mice.

			Monkey serum immune to: I (Brunhilde)						II (Lansing)						III (Monkey Leon)						Normal monkey serum					
			Cellular degeneration in days																							
Neutralization test in tissue culture	Virus		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
	I	Mahoney	—	—	—	—	—	—	—	+	+	+	+	+	—	+	+	+	+	+	—	+	+	+	+	+
	II	YSK	—	—	+	+	+	+	—	—	—	—	—	—	—	—	+	+	+	+	—	—	+	+	+	+
	III	Saukett	—	+	+	+	+	+	—	+	+	+	+	+	—	—	—	—	—	—	—	+	+	+	+	+

				I (Brunhilde)	II (Lansing)	III (Monkey Leon)	Normal monkey serum
Neutralization test in mice	I	Mouse Mahoney	Paralysis	0/8	6/8	8/8	7/8
			Incubation (days)	0	3.1	4	4.5
	II	Mouse MEF-1	Paralysis	10/10	2/10	9/10	9/10
			Incubation (days)	2.2	7	4.8	2.2
	III	Mouse Leon	Paralysis	8/9	10/10	0/10	9/10
			Incubation (days)	6	6	0	5

tion, lesions suggestive of poliomyelitis were found in the brain and spinal cord.

Intraspinal inoculation of a group of 4 and a group of 6 Syrian hamsters resulted in paralysis of half of each group, but 2 groups of cotton rats, similarly inoculated with a suspension that paralyzed 10 of 20 mice, have thus far resisted this virus. Intracerebral inoculation has thus far failed to paralyze any of the species of animals used.

There are some interesting differences between the Type I mouse adapted virus and mouse Types II and III. Type III mouse adapted virus does not paralyze hamsters and following serial tissue culture passages loses its virulence for mice. The MEF-1 Type II mouse virus, in adequate doses, infects mice readily by the intracerebral route as well as by the intraspinal and usually paralyzes most or all of the inoculated mice within 48 hours and produces symptoms in cotton rats.

Identity of the virus. At present, the immunologic evidence supporting the identification of this virus as poliomyelitis Type I rests primarily with the results of neutralization tests. These were performed on several occasions during the course of propagation and were conducted in both tissue cultures and mice. Table III illustrates the outcome of a typical test. On each occasion the virus was inhibited by Type I, but not by Types II and III, immune monkey sera prepared and previously tested in this laboratory for specificity (Table IV). In addition, sets of hyperimmune antisera obtained from two other poliomyelitis laboratories were also used to check the specificity of the virus. The results obtained with these sera confirmed the findings shown in Tables III and IV, adding further evidence that the mouse-adapted virus could be correctly classified as Type I. Results of cross immunization and cross infection experiments currently under way are as yet incomplete.

Discussion. In attempting to adapt Type I poliomyelitis virus to mice a number of methods were tested with view toward reducing the resistance of the experimental animals. These

included the use of cortisone, metabolites, exposure to cold, multiple routes of inoculation and other procedures. None of these were successful except the use of modified virus introduced intraspinally. This work illustrates the importance of the intraspinal route in mouse adaptation of poliomyelitis viruses, although the mechanism of pathogenicity by this route is not yet clear. With further passages it is anticipated that a better adaptation of the Type I poliomyelitis virus will be effected as was the case with Type III virus(5). It is especially significant that a virus strain which grows well in extra-neural tissues does not obviously infect when introduced into mice by various routes, including the intracerebral, and yet produces the typical poliomyelitic infection when injected directly into the spinal cord.

It is evident also from the data presented that the route of infection *per se* was not the only factor involved in the successful adaptation of this virus to mice. Obviously some alteration in the properties of the virus occurred in the course of tissue culture passage which, combined with intraspinal inoculation, permitted this adaptation. This may have been a quantitative change, a true mutation, or a temporary variation. It is possible also that this change may be associated with the unmasking of a component which enhances mouse virulence or the elimination of an inhibitor. It may be of significance that in the adaptation, this strain has lost its original virulence for monkeys.

While answers to certain questions must await further investigations which are currently in progress, preliminary tests indicate that this virus strain may be utilized adequately in the performance of relatively rapid neutralization tests in mice and tissue cultures.

This adaptation of the Type I poliomyelitis virus to rodents, when confirmed by other investigators, will provide the long-desired simplified tools with which to conduct, on a more extensive scale, the important field and laboratory studies previously hampered by tedious and expensive methods. Although primary isolation of poliomyelitis virus is not yet possible in the mouse, the adaptation of the 3 virus types to this animal will permit the performance of rapid, reproducible, and relatively inexpensive serologic and immunologic tests.

Summary. 1. The procedures employed in the successful adaptation of Type I (Mahoney) poliomyelitis virus to Swiss mice are described. This virus could also infect hamsters and C3H mice but not cotton rats, while its original virulence for monkeys was lost. 2. The use of the intraspinal route of inoculation and the selection of a mutant or variant of the virus is believed to be responsible for this adaptation. Other possible explanations are discussed. 3. With this attainment, all 3 poliomyelitis virus types have now been adapted to mice. This will permit more rapid progress in field and laboratory studies of poliomyelitis.

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