Adaptation of Type I Strain of Poliomyelitis Virus to Mice and Cotton Rats. (21061)

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The adaptation to mice and hamsters of Type I poliomyelitis virus, using the intraspinal route, was reported recently by Li and Schaefer(1). They were unable, however, to adapt their strain to cotton rats, and had no success with the intracerebral route. These authors claimed that continuous mouse passage of the strain reduced its intracerebral pathogenicity for the monkey(1).

The present paper describes the adaptation of a Type I strain of poliomyelitis virus to both mice and cotton rats using either intraspinal or intracerebral inoculation. Some virus sublines thus obtained, proved to be singularly apathogenic for monkeys when given by the intracerebral route.

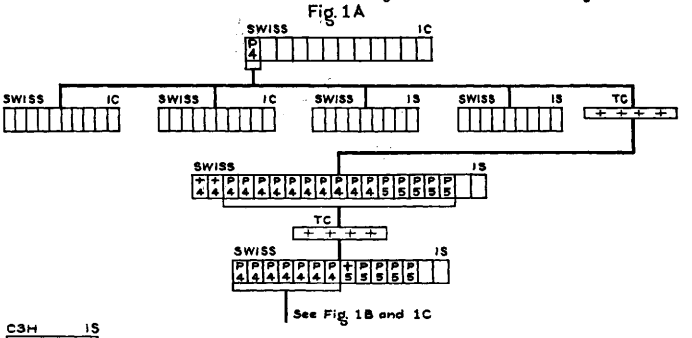
Materials and methods. Virus strains. The Sickle(2) strain was obtained from Dr. Albert Sabin, Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio, in the form of monkey spinal cord suspension representing the third monkey passage of the virus since its original isolation. The Mahoney(2) strain was procured through the kindness of Dr. Gordon Brown, Dept. of Epidemiology, School of Public Health, University of Michigan, in the form of monkey cord suspension representing their Pool III. Both strains were passaged once in monkey CNS, and the resulting spinal cord suspensions were then used to adapt these strains to tissue cultures. *Tissue cultures.* The technic was similar to that currently used in most laboratories(3,4). Fragments of monkey (Java) kidney tissue, embedded on the walls of roller tubes, were immersed in 199 nutrient medium(5). *Animals.* Swiss mice, cotton rats, and Java and rhesus monkeys were obtained from commercial dealers. The following inbred strains of mice were used: dba, C3H carrying the milk factor (C3H+), C3H free of the milk factor (C3H–), F-1 generations representing crosses between dba and C3H+, PRI and ICR (Swiss strain specially inbred at the Institute for Cancer Research, Fox Chase, Philadelphia, Pa.). All these strains were bred in this laboratory. Original stocks, with the exception of PRI, were obtained from Dr. T. Hauschka of the above Institute. The PRI mice were furnished by Dr. Albert Sabin of the University of Cincinnati. *Pathologic examination* of the central nervous system was performed usually 4-5 weeks after inoculation, using the method of Nissl in celloidin imbedded material. For quantification of cellular losses in the anterior horn of the spinal cord, Bodian's procedure(6) was used.

Experimental. Adaptation of the virus to PRI mice. Undiluted tissue culture fluid infected with the 8th tissue culture passage of Sickle strain was mixed in equal proportions with fluid infected with the 10th tissue culture passage of the Mahoney strain, and the mixture was injected intracerebrally into 12 Swiss mice. A single animal was found paralyzed on the 4th day after inoculation (Fig. 1A). A 10% suspension of its spinal cord was injected intraspinally into mice and inoculated into tissue culture. Although cytopathogenic effect was noted in the latter, no signs of sickness were observed in the injected mice. However, inoculation of tissue culture supernatant into mice intraspinally caused paralysis in 17 out of 19 animals (Fig. 1A). One more alternate passage between tissue cultures and mice resulted in 12 out of 14 animals becoming infected. The spinal cords of 7 paralyzed mice were made into a 10% suspension and inoculated intraspinally into groups of mice representing 7 different genetic strains. When and if inoculated animals became paralyzed, their spinal cords were removed, made into a 10% suspension and injected intraspinally into mice of the homologous strain. The results of the ex-

Adaptation of SM Virus to PR1 Mice

Equal Volumes Mahoney 10th TC Passage Plus Sickle 8th TC Passage

Fig. 1A



C3H IS P = C3H or other mouse strain injected intraspinally and found paralyzed

C3H IC P = C3H or other mouse strain injected intracerebrally and found paralyzed

TC + + + + = 4 plus cytopathogenic effect in monkey kidney culture

TC O = Complete lack of cytopathogenic effect in monkey kidney culture

Blank space inside of figure idicates no signs of illness

PP = Partially paralyzed IS = Intraspinal + = Dead

P = Completely paralyzed IC = Intracerebral

Fig. 1B*

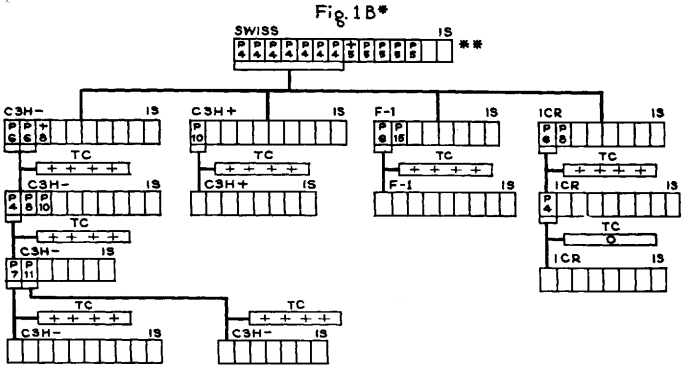
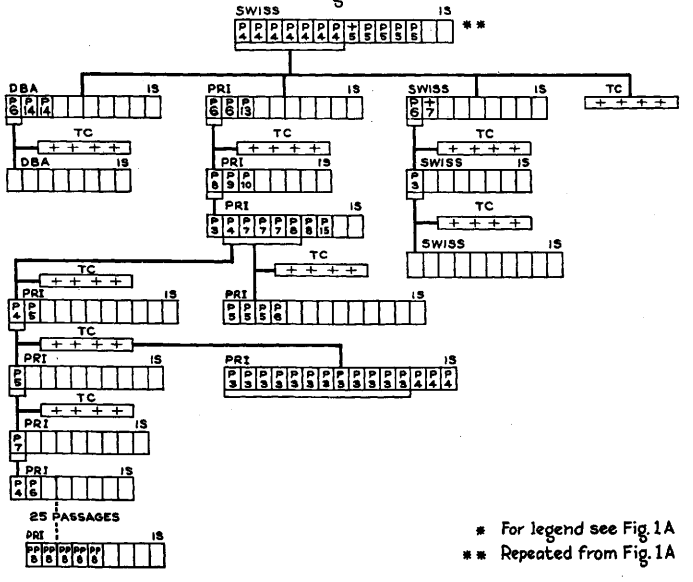


Fig. 1C *

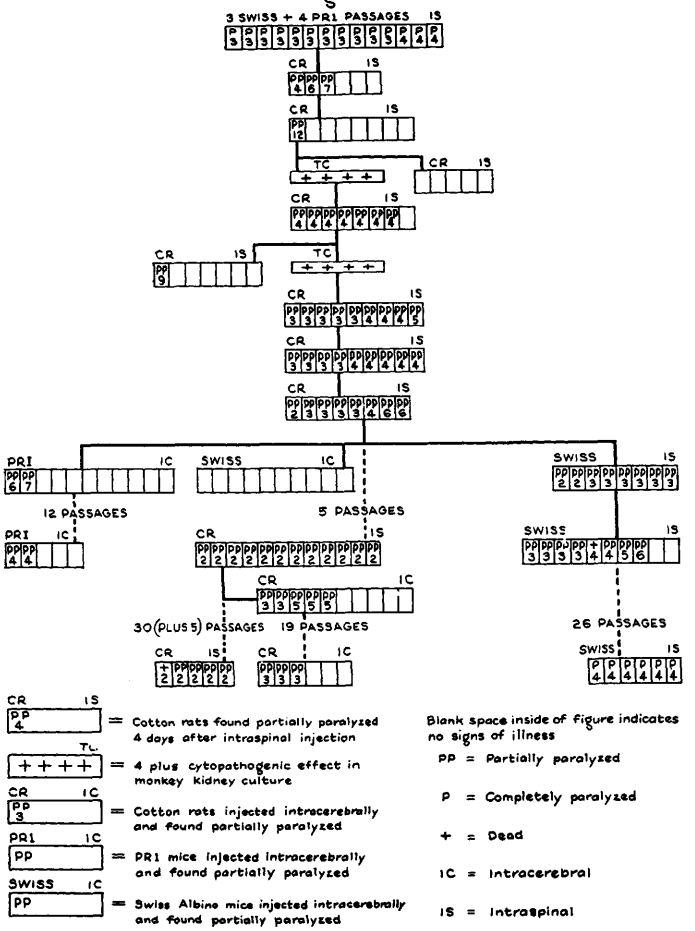


* For legend see Fig. 1A

** Repeated from Fig. 1A

Adaptation of SM Virus to Cotton Rats

Fig. 2



periments are shown in Fig. 1B and 1C. It may be seen from the paralysis ratio that the susceptibility of different breeds of mice was about the same at the first passage level. However, beyond the second passage the virus could be propagated in only 2 strains of mice: the C3H– (Fig. 1B) and the PRI (Fig. 1C). The infectivity faded away in the C3H-mice after the third consecutive passage, whereas the propagation of the virus in PRI mice was maintained in an unbroken sequence. Up to date, the virus has been propagated through 25 serial passages in PRI mice injected intraspinally with 10% suspensions of infected cord tissue. At this level the incubation period varies between 5 and 8 days, and usually more than half of the inoculated mice show signs of paralysis. The cytopathic titer of the virus in CNS of the mouse varies from $10^{-4.00}$ to $10^{-5.00}$, and one passage through tissue culture medium increases the pathogenicity of the agent for PRI mice.

Adaptation of virus to cotton rats. Material representing the seventh mouse passage (Fig. 2) was introduced intraspinally into 6 cotton rats. Three of these animals became paralyzed 4 to 7 days after inoculation, and their spinal cord tissue was injected into 8 cotton rats. As shown in Fig. 2, one of these animals sickened on the 12th day after inoculation and its cord tissue was inoculated into tissue culture tubes and intraspinally into five cotton rats. Although as noted in Fig. 2, cytopathic effect was observed in tissue cultures, no signs of sickness were elicited in the inoculated cotton rats. However, the

intraspinal inoculation of the tissue culture supernatant into cotton rats caused paralysis in 7 out of 8 animals. After one more alternate passage through tissue culture, the virus became adapted to cotton rats, and the resulting strain was maintained in an unbroken sequence in 3 main sublines.

Cotton rat passage subline. In one series (Fig. 2), the SM virus was propagated through 30 passages in cotton rats injected intraspinally. At this level the incubation period varied from 2 to 3 days, and all the animals inoculated intraspinally with a 10% suspension of spinal cord tissue became paralyzed. In a series not shown in the Figures material representing the 32nd cotton rat passage of the virus was injected *intracerebrally* into a group of cotton rats, and has been propagated in this fashion through 10 consecutive passages up to date. The incubation period in animals injected intracerebrally varies from 3 to 7 days, and usually 60-80% of animals injected with a 10% suspension of infected brain and spinal cord tissue become paralyzed. The other "intracerebral" series originated from the sixth intraspinal passage (Fig. 2). In this subline, the virus has been maintained through 19 consecutive intracerebral passages, using infected brain tissue as virus source. The incubation period is from 3 to 4 days, and almost all of the animals injected with a 10% suspension of brain tissue die from infection. In the first cotton rat series, virus representing the 32nd intraspinal cotton rat passage was adapted to Swiss albino mice injected intracerebrally. So far, the virus has been passaged without difficulty through 6 consecutive mouse passages using cord tissue as inoculum (not shown in the figure). The incubation time is from 3 to 6 days, and the mortality ratio is not uniform, although the series has been maintained in unbroken sequence.

Swiss albino mouse passage subline. Cotton rat cord suspension representing the 6th cotton rat passage of SM virus was injected into 8 Swiss mice intraspinally (Fig. 2). All of the mice became paralyzed on the third day after inoculation, and their spinal cord tissue was used as inoculum for another group of Swiss mice (Fig. 2). When 8 out of 10 animals in this group became paralyzed, their cord tissue was used to initiate a series of passages in mice which has been carried up to date through 26 consecutive generations. After intraspinal inoculation with a 10% suspension of mouse spinal cord tissue, mice show signs of paralysis on the third to fourth day. All animals died of infection.

TABLE I. Serological Identity of SM Strain Results of Neutralization Tests in Tissue Culture.

Passage	Dilution	No. of cytopathic units	Antiserum type	Ratio of TC tubes with cytopathic effect†
7 M + 1 CR*	1:10	N.T.	I	0/2
	1:100		I	0/3
	1:10		None	3/3
	1:100		None	3/3
+ 5 CR*	1:20	50000	I	0/3
			II	3/3
			III	3/3
			None	3/3
+ 41 CR*	1:10	5600	I	0/6
			II	6/6
			III	6/6
			None	6/6

M = mouse passage; CR = cotton rat passage.
* Plus one tissue culture passage.
† Denominator = No. of tubes inoculated with serum and virus; numerator = No. of tubes showing cytopathic changes.

PRI mouse passage subline (intracerebral series). This subline was initiated with starting material from the sixth cotton rat passage (Fig. 2) injected intracerebrally into mice of the PRI strain. As indicated in Fig. 2, this series has now been maintained for 12 consecutive passages. The incubation period varies from 4 to 6 days, but usually less than half of the inoculated mice show signs of paralysis.

Identification of the SM strain. Table I summarizes results of neutralization tests in tissue culture with SM virus representing three different passage levels. Antisera were prepared in this laboratory in rhesus monkeys following the technic described by Salk(7). These sera were found to be type specific when submitted to numerous neutralization tests prior to actual use for identification purposes. It may be observed from the results summarized in Table I that the SM virus retained the serological identity of a Type I strain throughout the series of passages in

TABLE II. Infectivity of Various Sublines of SM Virus.

Subline*	No. of passages in: Cotton rat i.s.	Cotton rat i.c.	Mouse Swiss	Mouse PRI	Tissue‡	Infectivity titer† in: Cotton rat	Mouse	Tissue culture
Cotton rat	10	16	—	—	B	3.50	2.10	5.50
					C	2.00	1.00	4.50
	32	6	—	—	B	3.00	1.40	4.50
					C	3.40	2.40	5.50
Swiss mouse	6	—	25	—	B	<1.00	<1.00	3.20
					C	2.50	1.50	4.20
PRI mouse	—	—	3	24	B	<1.00	<1.00	5.20
					C	<1.00	1.20	5.50

* For information concerning various sublines see text and also Fig. 1A, 1C and 2.
† Expressed as negative log to the base of 10.
‡ B = brain; C = cord.

mice and cotton rats. Five rhesus monkeys were inoculated intracerebrally with SM virus representing the third cotton rat passage (Fig. 2). Two of the animals were sacrificed while paralyzed. The other 3 were challenged 30 days later with 100 PD_{50} of Type I virus (Brunhilde strain, monkey cord tissue). None of these monkeys showed signs of paralysis, whereas 8 out of 9 non-immunized control monkeys became paralyzed.

Comparative infectivity of different sublines of SM virus for mice and cotton rats. As seen in Table II the infectivity of the 2 cotton rat sublines and that of the PRI mouse passage are similar when measured by the cytopathic effect in tissue culture, while the infectivity of the Swiss mouse passage line is lower. The virus content of brain was lower than that of spinal cord in all sublines except the one representing the cotton rat series with the highest—19—number of intracerebral passages. Virus maintained solely by serial passage through mice of the PRI line is apathogenic for cotton rats, of low virulence for mice, and of high infectivity for tissue culture. Virus maintained by serial passage through Swiss mice after 6 passages through cotton rats retained its infectivity for cotton rats (spinal cord tissue). Brain tissue material representing two virus lines which are maintained through intraspinal inoculation of mice is non-pathogenic for either cotton rats or mice, although the fact that it contains virus is indicated by cytopathic effect in tissue culture. Conversely, the virus content of brain tissue of cotton rats injected intracerebrally can be determined by titration in either mice or cotton rats. The virus titer, however, apparently increases with the number of intracerebral transfers.

Comparative pathogenicity of the various sublines for monkeys. As seen in Table III, the SM subline propagated in cotton rats was as virulent for monkeys as the original unadapted strain, as judged from the clinical manifestations and the pathologic findings (Table IV). The cellular loss was apparently irrespective of the virus dilution. The *Swiss mouse subline* showed little virulence for monkeys (Table III). At the 12th passage level none of the 5 monkeys inoculated showed clinical manifestations. Pathologic examination disclosed no appreciable cellular loss and no evidence of inflammatory changes. However, at the 22nd passage level 2 monkeys out of 15 injected with different dilutions of the virus became paralyzed; loss of cells in the anterior horn was 62 and 20% respectively. The other 13 monkeys showed no pathologic evidence of poliomyelitis. A moderate degree of paralysis was also observed in 2 rhesus monkeys representing a group of 12 animals inoculated with dilutions of tissue culture supernatants infected with the 35th Swiss mouse passage of the virus. The other 10 monkeys showed neither clinical nor pathologic evidence of poliomyelitis. The *PRI mouse subline,* as seen in Table III, shows no virulence for either rhesus or cynomolgus monkeys. In 3 separate experiments, material representing different passage levels of the virus failed to elicit signs of illness in any

TABLE III. Virulence of SM Rodent-Adapted Strain of Poliomyelitis (Type I) for Monkeys.

Inoculum: Host*	Passage No.	PD_{50} titer§ in: Mice	Cotton rats	TD_{50}‖ titer	Paralytic ratio of monkeys injected with dilutions of virus: 10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Monkey species	Route of inoculation
Cotton rat	12 + 11 TC	2.75	N.T.	6.70	2/4	4/4	4/4	3/4	1/4	0/4	0/4	Java	Intracer.
	16	2.80	N.T.	5.50	—	8/10	1/2	2/2	1/2	2/2	—	Rhesus	
Mouse,† Swiss strain	12	2.75	N.T.	5.50	—	0/5	—	—	—	—	—	"	
	22‡	1.00¶	2.15	4.50	—	2/4	0/3	0/2	0/2	0/2	0/2	"	
	35 + 1 TC	N.T.	N.T.	6.00	1/3	1/3	0/3	0/3	—	—	—	"	
Mouse, PRI strain	26 + 8 TC + 1 mouse	1.50		5.20	—	0/4	0/4	—	—	—	—	"	
	22 + 1 TC‡ + 1 mouse	2.00	<1.00	5.20	—	0/4	0/4	0/4	0/4	0/2	—	Java	Intrav.
	28 + 1 TC + 1 mouse	1.20	N.T.	3.20	—	0/4	0/4	—	—	—	—	"	Intrav.
					—	0/4	0/4	—	—	—	—	"	Intracer.
					—	0/4	0/4	0/4	0/4	0/4	—	Rhesus	Intrav.
					—	0/4	—	—	—	—	—	"	

* Virus previously passaged through 3 Swiss Albino and 4 PRI mice. † Six passages through cotton rats preceded the mouse passage line.
‡ Used for oral administration to human subjects. § Expressed as negative log to base of 10. ‖ Cytopathogenic effect in tissue culture.
¶ Mice found paralyzed at 10^{-1} dilution.
TC = Tissue culture passage. N.T. = Not tested.

TABLE IV. Histopathologic Changes (Cell Loss) Observed in Spinal Cord Tissue of Cynomolgus Monkeys Injected I.C. with Dilutions of SM Strain.*

Monkey	Virus dilution	Cell loss, % Cervical	Cell loss, % Lumbar
30–33	10^{-1}	49	46
30–34	"	78	82
30–36	"	35	37
30–38	"	74	58
30–39	"	37	70
30–43	10^{-2}	60	35
30–45	10^{-3}	46	44
30–46	"	80	41
30–47	10^{-4}	60	65
30–49	10^{-5}	29	26

* Representing 16th cotton rat passage (see Table III).

of the 54 intracerebrally injected monkeys. Histologically, in none of these animals was there found appreciable loss of neurons or inflammatory reaction. The avirulence of the virus was further confirmed in the last experiment (Table III) when groups of cynomolgus or rhesus monkeys were injected intravenously with infected mouse cord suspension. This route of inoculation was chosen since it made possible the administration of a large quantity (5 ml of 10% suspension of mouse spinal cord) to an animal, and because monkeys had previously been observed to be especially susceptible to that particular route of infection. As seen in Table III, none of the intravenously injected monkeys showed signs of poliomyelitis, and the results of histopathologic examinations were equally negative.

The attenuation of the SM virus may have occurred as a direct result of selection of avirulent (for monkeys) mutants in the course of propagation in the central nervous system of the PRI mouse. An alternative hypothesis is that while the genetic units of the SM strain remained undisturbed, characteristics of the virus population changed under the direct influence of the cellular ambient (PRI mouse CNS) in which the virus propagated. If the latter hypothesis concerning non-genetic changes be correct, then passage of the PRI subline in another host cell would induce changes in its pathogenic properties. This did not occur, however, when virus representing the 26th PRI mouse passage (Table III)

was passaged through eight consecutive passages in cynomolgus renal epithelium cells grown in tissue culture. It would seem, therefore, that the attenuation of SM virus might well be considered as directly attributable to the outgrowth, in the course of passages through PRI mice, of selective mutants avirulent for monkeys.

Summary. A mixture of 2 strains of Type I poliomyelitis virus was adapted to the PRI strain of mice and to cotton rats. The virus could be propagated in certain sublines when either the intraspinal or intracerebral route of inoculation was used. The infectivity of different sublines for mice and cotton rats varied, but the cytopathic titer in tissue culture was of similar magnitude. The passages in PRI mice resulted in a subline completely avirulent for rhesus and cynomolgus monkeys.

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Received May 3, 1954. P.S.E.B.M., 1954, v86.

Administration of an Attenuated Type I Poliomyelitis Virus to Human Subjects. (21062)

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The preceding paper(1) describes the adaptation of a Type I strain of poliomyelitis virus to mice and cotton rats, and presents evidence of attenuation of the virus for monkeys injected intracerebrally. It has been previously reported that a Type II rodent adapted strain of similarly attenuated properties, when administered orally to 85 nonimmune human subjects, produces persistent immune response(2-4). This report describes the results of feeding Type I mouse adapted strain to 2 human subjects, and a mixture of Type I and Type II strains to one subject. These 3 individuals were affected with fatal neoplastic disease, and none had antibodies against the viruses fed.

Materials and methods. Virus. Two pools of infectious material were used for individuals who received only Type I virus: one represented the 22nd passage of the Swiss mouse subline of the combined Sickle and Mahoney strains(1), and the other represented the 22nd + one tissue culture + one mouse passage of the PRI mouse passage line. As stated elsewhere(1), both preparations had been found to have little or no pathogenicity for monkeys injected intracerebrally. Each feeding dose consisted of one ml of a 20% suspension of infected mouse spinal cord tissue diluted in one ounce of milk. The third individual received 4 ml of a virus suspension consisting of equal parts of the SM strain (Type I) and the TN strain (Type II). The SM strain represented the 28th PRI mouse + TC + one mouse brain passage of the virus(1), and the TN strain the 8th mouse brain passage of the virus(2).

Clinical observations, collections of blood and stool specimens. These were conducted according to methods described in detail before(2,3).

Method of virus assay. For determination of the presence of virus in stools or blood, 6 tissue culture (cynomolgus monkey renal epithelium) tubes were used for each specimen according to the method described by Horstmann(5). If all 6 tubes showed no cytopathic effect, 2 blind passages were made