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Poliomyelitis. I. Propagation of the MEF1 Strain of Poliomyelitis Virus in the Suckling Hamster.* (19927)

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The work reported here was initiated originally with the thought of developing a high titered virus which could be used to produce a more practical complement-fixation antigen for the diagnosis of poliomyelitis. Since the Syrian golden hamster appears to be less prone to spontaneous virus infections than the Swiss white mouse it was chosen for the serial transmission of the MEF1 strain(1,2), Type 2 poliomyelitis virus. While our work was in progress Casals, Olitsky and Anslow(3,4) and Selzer, Sacks and van den Ende(5) adapted the MEF1 strain of poliomyelitis virus to newborn mice, but no modification in virulence of the virus for primates was reported. During the course of our work, however, it was conceived that continuous passage of the virus in young hamsters might result in the production of higher concentrations of virus than are ordinarily attained in mice and that through adaptation or fixation for the species, in the Pasteurian sense, the virus might broaden its host range and thereby lose virulence for those species ordinarily more susceptible. Could these hopes be realized without loss of antigenicity by the virus, material progress would be made in the development of a practical diagnostic antigen as well as immunizing agents in the form of inactivated or live virus vaccines. Adaptation to new hosts, especially the chick embryo, would further expand the possibilities of research and greatly simplify the preparation of diagnostic and prophylactic antigens.

The present report describes the development of a passage strain of MEF1 poliomyelitis virus carried in 7- to 10-day-old suckling hamsters and of another strain carried in 2- to 4-day-old hamsters.

Materials and methods. Virus strains. The MEF1 Lansing type (Type 2) strain of human poliomyelitis virus was obtained as a 50% glycerine-water mouse brain suspension through the kindness of Dr. Peter K. Olitsky and Dr. Jordi Casals of the Rockefeller Institute for Medical Research, New York, N. Y. The strain was passed by intracerebral inoculation for 9 passages in Swiss white mice and was thereafter carried intracerebrally in 6- to 8-week-old Syrian golden hamsters. A virus pool was prepared from the nervous tissues harvested from the 30th to the 34th hamster passages in the following manner: the brain and spinal cord tissues were aseptically removed from paralyzed hamsters and were thoroughly ground in a TenBroeck grinder; a 10% tissue suspension of the

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† Sodium chloride 9 g; potassium chloride .3 g; calcium chloride .2 g; sodium carbonate .2 g per liter of solution.

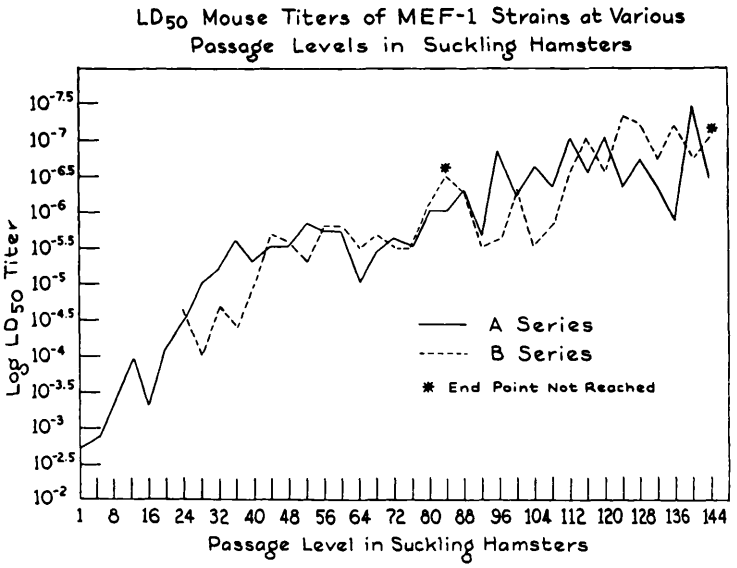

FIG. 1.

ground tissues was made in sterile Ringer's solution;† the suspension was centrifuged for 30 minutes at 2500 rpm in a size 1 International anglehead centrifuge; the supernate from this preparation was used to initiate the A series of passages in suckling hamsters. The same method of preparation was used in making tissue suspensions for subsequent passages in hamsters. A *virus pool* prepared from the 64th hamster passage of the MEF1-A strain described below was used in serum neutralization tests. The *Wallingford strain*(2), Type 2 poliomyelitis virus, shown in Table II, represented the first monkey passage of a virus suspension designated WLFD IV G981, which was received from Dr. Howard A. Howe, of the Poliomyelitis Research Laboratory, the Johns Hopkins University, Baltimore, Md. The *Brunhilde strain*(2), Type 1 poliomyelitis virus, represented the 3rd monkey passage of a virus suspension which was also obtained through the courtesy of Dr. Howe.

Passage method. A 0.03 ml dose of inoculum was injected intracerebrally in suckling hamsters in the A series passages. Passage in the 2- to 4-day-old hamsters of the B series was made with a 0.02 ml dose intracerebrally. In the later passages Ringer's solution was replaced by isotonic sodium chloride solution as a suspending medium. Brain and spinal cord tissues were harvested for passage. *Immune sera.* An anti-Lansing immune hamster serum was prepared by hyperimmunization of hamsters which had survived intramuscular injection of the MEF1 strain. A series of intramuscular injections starting with 0.25 ml of a 10^{-4} dilution of virus tissue suspension and ending with 0.5 ml of a 10^{-1} dilution was given at weekly intervals. An anti-Lansing immune monkey serum was prepared by hyperimmunization of a convalescent monkey which had been intracerebrally injected with the WW, Type 2 strain of poliomyelitis virus(6). The Armstrong strain(2) of Lansing type virus was used by the intramuscular route for hyperimmunization.

Neutralization tests. Undiluted hamster or monkey serum was mixed with an equal volume of a 20% virus tissue suspension or tenfold dilutions thereof, and the mixtures were incubated at room temperature for 90 minutes. Groups of 4 to 5 mice weighing 12 to 14 g were injected intracerebrally with 0.03 ml of the serum-virus mixtures. Daily observations were recorded for 21 days. Protective indices were calculated on the basis of the difference between the log LD_{50} titers of the virus in the presence of normal and immune serum. The neutralizing potency of the chimpanzee sera was calculated by the Reed and Muench method(7) on the basis of serial

TABLE I. Identification of the Two Hamster-Adapted MEF1 Strains of Poliomyelitis Virus. Serum neutralization tests.

Virus strain, passage series	Hamster passage	Serum	Serum dilution	Mortality ratio of mice inoc. intracer. with serum and dilutions of virus 10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	LD_{50} titer of virus	Protective index
MEF1-A	83	Normal hamster	Undil.	5/5	5/5	5/5	2/5	0/5	0/5	$10^{-4.83}$	676
		Immune " *	Undil.	2/5	0/5	0/5	0/6	1/3		$10^{-2.00}$	
	130	Normal monkey	1:8		5/5	4/4	5/5	5/5	1/5	$10^{-6.63}$	426
		Immune " †	1:8		5/5	2/5	1/5	0/5	0/5	$10^{-4.00}$	
MEF1-B	126	Normal monkey	1:8		5/5	5/5	5/5	5/5	2/5	$10^{-6.83}$	1440
		Immune " †	1:8		4/5	2/5	0/5	0/5	0/5	$10^{-3.67}$	

* Lederle hyperimmune hamster serum.
† " " monkey " .

dilutions of serum tested against a predetermined number of LD_{50} doses of virus.

Experimental. Series A hamster passage. The A passage series was initiated on July 16, 1948, in 7- to 10-day-old suckling hamsters by intracerebral inoculation of hamster pool virus. Brain and spinal cord tissues were harvested at 2 to 4 days when the hamsters showed paralytic symptoms, usually in one of the forelegs. Passages were routinely made in one or more litters of suckling hamsters. The incubation period gradually shortened so that by the 121st passage symptoms began to appear as early as 24 hours after intracerebral inoculation. This young hamster passage sequence, designated the A series, has been maintained for 150 consecutive generations and is still in progress. The inoculum for each passage was titrated intracerebrally in tenfold dilutions in groups of 4 to 5 Swiss mice and the titers for every 4th passage, calculated by the method of Reed and Muench(7), are presented in the accompanying chart. There was a gradual increase in the concentration of virus demonstrated in the nervous tissues. From the 96th hamster passage onwards the titers rarely fell below $10^{-6.5}$ and were often found to be $10^{-7.0}$ or greater. In the 140th passage, for example, the titer reached $10^{-7.4}$.

Series B hamster passage. At the 23rd passage level in 7- to 10-day-old hamsters a subpassage series was started on February 23, 1949 in 2- to 4-day-old hamsters. This series was labeled "B" and the first such passage was designated B-24. The intracerebral dose was reduced to 0.02 ml in deference to the size of the baby hamsters but otherwise the passage technic was the same as that used in the A series of hamster passages. Excluding the 23 passages in 7- to 10-day-old hamsters, this substrain (series B) has been maintained for 116 consecutive passages in 2- to 4-day-old hamsters and is still in progress. At each passage level the inoculum was titrated in mice as in the A series and the LD_{50} titers for every 4th passage are presented in the accompanying chart. The titers attained in this series are equally as high as those demonstrated in the A series.

Identification of the passage virus. The details of the mouse neutralization tests are summarized in Table I. The 83rd hamster passage virus of the A series was tested against MEF1 immune hamster serum. Similarly, the 130th passage of the A series was tested against the Lederle anti-Lansing immune monkey serum. This same immune monkey serum was used against the 126th hamster passage virus of the B series. The data obtained in these three neutralization tests clearly indicate that the virus strains in the A and B passage series is Lansing type human poliomyelitis virus. The identity of the passage strains as poliomyelitis virus is further implied by the ability of the Wallingford strain, which is a Lansing type of human poliomyelitis virus, to elevate the neutralizing titer of the chimpanzee sera for the MEF1-A strain of virus (Table II).

Virulence tests in monkeys and chimpanzees. At the 54th passage level of the A strain of virus, 2 Rhesus monkeys were injected intramuscularly with 5 ml each of a 20% suspension of pooled hamster brain and cord tissue

TABLE II. Oral Administration of Poliomyelitis Virus to Chimpanzees. Neutralization tests at various intervals after feeding.

	Administration of poliomyelitis virus					Serum taken .... days later	Dilutions of serum neutralizing various LD_{50} doses of MEF1-A strain virus			
	Virus fed	LD_{50} titer in mice	Suspension, %	Vol., ml	Date fed			24 LD_{50}	18 LD_{50}	6 LD_{50}
Chimp. No. 1						0	Pre-feeding	<1:2	<1:2	<1:2
	B-82*	$>10^{-6.5}$	20	10	7/26/50	42	Post-1st feeding	—	1:16	1:238
	B-84	$>10^{-6.5}$	20	5	9/ 7	140	Post-2nd feeding	—	1:5	1:53
	B-94	$10^{-5.8}$	20	5	1/26/51	14	Post-3rd feeding	1:378	1:436	1:3540
	Brun.†	—	20	10	2/12	67	Post-Brun.	1:138	1:862	1:2380
	Wall.‡	—	10	7	4/23	29	Post-Wall.	—	1:654	1:3540
								30 LD_{50}	24 LD_{50}	10 LD_{50}
Chimp. No. 2						0	Pre-feeding	<1:2	<1:2	<1:2
	B-82*	$>10^{-6.5}$	20	10	7/26/50	42	Post-1st feeding	—	—	1:37
	B-84	$>10^{-6.5}$	20	5	9/ 7	140	Post-2nd feeding	1:3	—	1:76
	B-94	$10^{-5.8}$	20	5	1/26/51	14	Post-3rd feeding	1:4	1:158	1:111
	Brun.	—	20	10	2/12	67	Post-Brun.	1:5	1:662	1:258
	Wall.	—	10	7	4/23	29	Post-Wall.	1:14	—	1:798

* B-82 = 82nd passage in suckling hamsters, B series, MEF1 poliomyelitis virus.
† Brun. = Brunhilde, Type 1 poliomyelitis virus.
‡ Wall. = Wallingford, Type 2 " "

which had a mouse LD_{50} titer of greater than $10^{-6.5}$. Both animals became paralyzed and died showing typical symptoms of poliomyelitis. At the 60th passage level of the B series of virus, 7 Rhesus monkeys were injected intracerebrally with 1 ml each of a 20% pooled hamster brain and cord tissue suspension having a mouse LD_{50} titer of $10^{-5.8}$. One of the 7 monkeys became paralyzed but survived, whereas the remaining 6 animals remained symptom-free. Similarly, 13 monkeys were injected intracerebrally with 1 ml each of a pooled hamster brain and cord tissue suspension of the 64th passage of the B series of virus, having an LD_{50} titer of $10^{-5.5}$ in mice. Two of the monkeys developed partial paralysis; two others became completely paralyzed but 9 remained symptom-free.

The apparently reduced virulence of the B series passage virus for Rhesus monkeys prompted a feeding test in chimpanzees. Accordingly, two chimpanzees were bled and then given feedings of 20% brain and cord suspensions representing the 82nd, 84th and 94th hamster passages of the B series of virus. The dates of feeding, volume and mouse LD_{50} titers of the suspensions fed are given in Table II, together with the serological findings. Serum samples were collected after each feeding of the B series virus and again following the feeding of Brunhilde and Wallingford virus suspensions. No virus was recovered from the stool samples collected after the feeding of the B series hamster passage virus, but Brunhilde virus was recovered by the inoculation of monkeys with stool samples collected from the chimpanzees 7, 10 and 14 days after being fed. Similarly, virus was recovered from stool samples collected 11 and 14 days after the feeding of Wallingford virus. The chimpanzees remained free of nervous symptoms throughout the course of the experiment.

The post-feeding serum samples, together with the samples obtained prior to feeding, were tested against the 64th hamster passage virus of the A series by mouse neutralization tests. The results, summarized in Table II, clearly indicate that the series B virus is capable of stimulating a systemic immune re-

sponse when administered by the gastrointestinal route, and that when given orally the strain is lacking in virulence for the chimpanzee. It is of further interest that the feeding of the Brunhilde and Wallingford strains of virus failed to induce symptoms in the treated animals and that antibody titers for the A series passage virus increased following the feeding of these heterologous strains. This observation takes on added interest and significance in the light of the recent reports of Casals *et al*(8) and Sabin (9) that Brunhilde type infection in man may be accompanied by the appearance of heterotypic Lansing type complement-fixing and neutralizing antibodies in the convalescent serum.

Infectivity by the intramuscular route in young mice. It was observed that both the A and the B series passage strains of MEF1 hamster-adapted virus would induce paralysis and death in 12 to 14 g white Swiss mice, which were inoculated by extraneural routes. This property, which has been observed by others(5,10), suggested the utility of these strains for the rapid assay of anti-Lansing type poliomyelitis antibodies. In a preliminary experiment it was found that the injection of 0.25 ml of gamma globulin in one flank of mice followed immediately by injection into the other flank of 0.25 ml of a 10% suspension of the nervous tissues harvested from the 121st hamster passage of the B series left 6 of 6 mice unaffected, while 6 control mice died when given virus alone. Table III summarizes the results of an experiment designed to test the duration of the passive immunity induced in weanling mice by administration of immune serum globulin of placental origin, and Table IV gives the results of a titration of a sample of commercial immune serum globulin for antibody content. The results illustrate the utility of this strain of virus for assay purposes and at the same time indicate the antigenic affinity of the passage virus for antibodies which occur in commercially available immune serum globulin.

Discussion. Continuous passage of the MEF1 strain (Lansing type, Type 2) of poliomyelitis virus in suckling hamsters by the intracerebral route has resulted in the development of two substrains of virus that exhibit properties which are markedly different from those of the parent strain. In conformity with the observations of others(3,5) who have worked with the MEF1 strain of poliomyelitis virus in young animals, it was noted that the incubation period shortened and that the concentration of virus in the nervous tissues increased with progressive serial passages in suckling hamsters. By harvesting the brain and cord tissues as early as 24 hours after inoculation, when the first symptoms of illness appeared, titers as high as $10^{-6.5}$ to $10^{-7.0}$, or even greater, were attained with considerable regularity. Virus concentrations of this magnitude have greatly facilitated the development of a complement-fixing antigen and an inactivated or killed virus vaccine, both of which are under investigation at the present

TABLE III. Protective Action in Mice of Immune Serum Globulin (Placental Origin) Against the MEF1-B Strain Poliomyelitis Virus.

Interval between treatment and challenge	Mortality ratio*	
	Treated mice	Untreated mice
24 hr	1/10	6/8
48	0/10	9/10
72	1/10	4/10
96	0/10	8/10
7 days	0/10	7/10
14	0/9	8/10
28	4/6	5/8

* Mortality ratio = No. of mice developing symptoms/No. surviving without symptoms.

TABLE IV. Neutralization Test in Mice with MEF1-A Virus and Immune Serum Globulin.

Material inj.	Globulin dilution	LD_{50} titer of virus
Virus* + immune serum globulin†	1:4	$10^{-1.83}$
	1:8	$10^{-2.68}$
	1:16	$10^{-3.17}$
	1:32	$10^{-2.68}$
	1:64	$10^{-3.31}$
	1:128	10^{-3}
	1:256	$10^{-3.15}$
	1:512	$10^{-3.63}$
Virus* + saline		$10^{-6.17}$
Virus* + normal rabbit serum‡		$10^{-5.32}$

* Virus = 110th hamster passage A series.

† Immune serum globulin = Lederle Lot #2167-82A.

‡ Normal rabbit serum contained 1:10000 merthiolate.

time. In this connection it should be noted that our data agree in general with those reported recently by Casals *et al.*(11,12).

The B series substrain of the MEF1 virus has a remarkably high degree of virulence for 2- to 4-day-old suckling hamsters as well as Swiss white mice, but it apparently has lost in great measure its pathogenicity for the Rhesus monkey. While the feeding experiment in chimpanzees with the B substrain of virus was exploratory only, it is of interest to note that in the absence of clinical signs of disease or demonstrable virus in their feces, both chimpanzees developed neutralizing antibodies for the A substrain hamster passaged MEF1 virus. Furthermore, when subsequently fed the heterotypic Brunhilde virus (Type 1) and the homotypic Wallingford virus (Type 2), both chimpanzees became symptomless excretors of these virus strains and showed an anamnestic antibody response for MEF1-A substrain of virus. Should the extensive work now in progress confirm the preliminary evidence that the B substrain of hamster passaged MEF1 virus is avirulent for primates, that it does not induce a carrier state but does immunize when administered by the oral route, material progress will have been made towards producing a practical living virus vaccine.

The high LD_{50} titers attained in suckling hamsters by these two substrains of MEF1 virus may be assumed to favor the appearance and capture of variants differing from those of the original virus clone(13) when methods appropriate for the demonstration of such variants are used. In the accompanying papers by Roca-Garcia(14) and Cabasso(15) *et al.* evidence is presented which suggests that a mutant of poliomyelitis virus capable of growth in the chick embryo appeared between the 109th and the 115th hamster passage of the MEF1-A virus. This evidence of broadened host range gives additional promise of the eventual development of an immunizing agent which may be both immunogenically effective and clinically acceptable.

Summary. 1. The MEF1 strain of poliomyelitis virus (Lansing type, Type 2) has been adapted to suckling hamsters using the intracerebral route of inoculation, whereby two substrains of virus have been developed which show a much shortened incubation period and a markedly increased concentration of virus in the nervous tissues. The strains adapted to suckling hamsters have been found to cause paralysis after intramuscular or intraperitoneal inoculation into 21- to 28-day-old mice. 2. Intracerebral inoculations in 20 Rhesus monkeys suggest the MEF1-B substrain of virus has become somewhat reduced in virulence for primates. Two chimpanzees which were fed nervous tissue suspensions of the B substrain remained symptom-free, developed neutralizing antibodies and failed to excrete virus in their stools.

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