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Injection of Mouse Adapted and Egg Adapted Poliomyelitis-like Virus into White Rats.

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During the past year we have cultivated 3 strains of mouse poliomyelitis virus in embryonated eggs. These are a highly mouse-virulent variant derived from the SK strain, the MM strain, and a highly mouse virulent variant derived from the Lansing strain. These were obtained from Dr. C. W. Jungeblut. At the same time we were not successful in cultivating the well known mouse adapted Lansing strain (obtained from Dr. Charles Armstrong) in embryonated eggs. Results of our studies on the immunology of these strains have recently been summarized.[1]

In experimental tests of host susceptibility of the highly virulent mouse strains (*i.e.* intracerebral doses of 0.03 cc of dilutions of 10^{-5} to 10^{-6} being fatal to mice), we injected a group of white rats of about 100 g weight each intracerebrally with 0.1 cc doses of 10^{-1} dilution of mouse brain SK virus. At one week after injection these rats appeared normal with the exception of slight loss of weight. They were sacrificed at this time and the brains removed for virus titrations intracerebrally in mice. Duplicate mice which received 0.03 cc of virus dilutions of 10^{-1} to 10^{-5} inclusive, and also one of 2 mice on a dilution of 10^{-6} died in either 3 or 4 days. A single mouse on a dilution of 10^{-6} survived. These results indicated that while rats exhibited practically no symptoms on first attempted passage of the mouse adapted high SK strain, their brains contained this virus in top titer. Top titers are also obtained by inoculating 0.1 cc of all decimal dilutions of virus up to 10^{-5}. Daily increase of virus is demonstrable and easiest to show following doses of 10^{-3}, 10^{-4} and 10^{-5}. This phenomenon resembles that observed by Hirst[2] when egg adapted strains of influenza virus are passed for the first time in mice which remain asymptomatic although their lungs may contain influenza virus as high in titer as is ever obtained.

In a second experiment we injected strain MM into 6 rats using doses of 0.1 cc of 10^{-1} dilution of mouse brain virus intracerebrally. One week after injection 3 rats were sacrificed for virus titration. A pair of mice receiving 0.03 cc of a single dilution of 10^{-4} of this rat brain virus both died within 3 days. No other mice were tested. The three remaining rats appeared normal 3 weeks after injection, and at this time were bled for tests of neutralizing antibody. The results are shown in Table I and indicate that the inapparent infection resulted in the production of rather strong neutralizing antibody when tested as has been described.[1]

In a third experiment we used virus strain MM in the shape of embryonated egg passage 4. Intracerebral doses of 0.1 cc of a 10^{-1} dilution of the chick embryo virus were injected intracerebrally into rats of about 100 g weight each. Five days after inoculation, part of the groups was sacrificed for virus which titered 10^{-4} intracerebrally in mice. (Incidentally 3 of these rats which were saved appeared healthy and normal one month after inoculation). The MM virus obtained from the sacrificed rats was used for immunization of 2 rabbits. These animals received 2.0 cc of 10^{-1} dilution of active virus intradermally twice weekly, and were bled one week after the tenth and final injection. The rabbit antiserum was then used in neutralization tests in mice against the highly mouse virulent SK virus, MM virus, and the highly mouse virulent Lansing strain. The results of these tests are shown in Table II. It is apparent that rabbit MM antiserum prepared by in-

[1] Powell, H. M., and Jamieson, W. A., *Jour. Inf. Dis.*, in press.

[2] Hirst, G. K., *J. Exp. Med.*, 1947, **86**, 357.

TABLE I.
Neutralization Tests in Mice of Serum from Rats Bled 3 Weeks After Intracerebral Injection of MM Virus.

Dilutions of MM mouse brain virus	Serum used for neutralization test					
	Immune serum			Normal serum		
	i.c.	i.p.	i.n.	i.c.	i.p.	i.n.
10-1	3,3	S,S	6,11	3,3	3,3	4,4
10-3	3,3	S,S	S,S	3,3	4,4	5,6
10-5	S,S	S,S	S,S	3,4	6,S	S,S
10-6	S,S	S,S	S,S	7,S	S,S	S,S

Legend: i.c., i.p., and i.n. indicate respectively intracerebrally, intraperitoneally, and intranasally. Each figure indicates day of death of one mouse. S indicates survival 14 days.

jecting rabbits with rat brain MM virus from asymptomatic rats brings about neutralization of the homologous MM virus and also the other two strains, namely high SK and high Lansing.

In further neutralization tests of this rabbit MM antiserum against the well known "low" Lansing virus, we observed no neutralization whatever. These tests were done by methods indicated in Table III of reference 1, and 10 mice were used on the immune serum plus virus mixture, and 10 mice were used on the normal serum plus virus mixture. At the end of 30 days 9 mice in the immune serum group and 8 mice in the normal serum group had died. The other mice survived.

Conclusions. 1. Strains of highly mouse virulent poliomyelitis virus as transmitted in mice or in embryonated eggs may be transmitted to rats without symptoms but with the development of virus of high titer in the brain in the first passage.

2. Such rat brain virus may be used to immunize rabbits effectively, and the antiserum so obtained neutralizes homologous and heterologous strains of the "high" variety, namely MM, high SK, and high Lansing.

3. The above antiserum does not neutralize the well known "low" Lansing strain of poliomyelitis virus.

TABLE II.
Neutralization Tests in Mice of Serum from Rabbits Immunized with Active Rat Brain MM Virus from Asymptomatic Rats.

Mouse brain virus used	Dilutions of virus	Serum used for neutralization					
		Immune serum			Normal serum		
		i.c.	i.p.	i.n.	i.c.	i.p.	i.n.
High SK	10-1	0	0	0	0	0	0
	10-3	3,S	S,S	S,S	3,3	3,4	4,4
	10-5	S,S	S,S	S,S	3,3	4,4	7,9
	10-7	S,S	S,S	S,S	S,S	0	0
MM	10-1	S,S	S,S	S,S	2,2	3,3	6,6
	10-3	S,S	S,S	S,S	3,3	4,4	7,S
	10-5	S,S	S,S	S,S	4,4	4,S	S,S
	10-7	S,S	S,S	S,S	S,S	S,S	S,S
High Lansing	10-1	3,6	S,S	S,S	2,2	3,3	3,3
	10-3	S,S	S,S	S,S	3,3	4,5	S,S
	10-5	S,S	S,S	S,S	3,3	6,S	6,S
	10-7	S,S	S,S	S,S	4,5	S,S	S,S

Legend: i.c., i.p., and i.n. indicate intracerebrally, intraperitoneally, and intranasally respectively. Each figure indicates day of death of one mouse. S indicates survival 14 days. 0 indicates not done.